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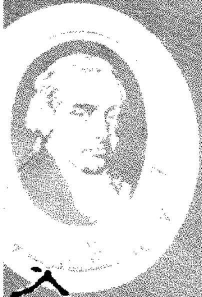

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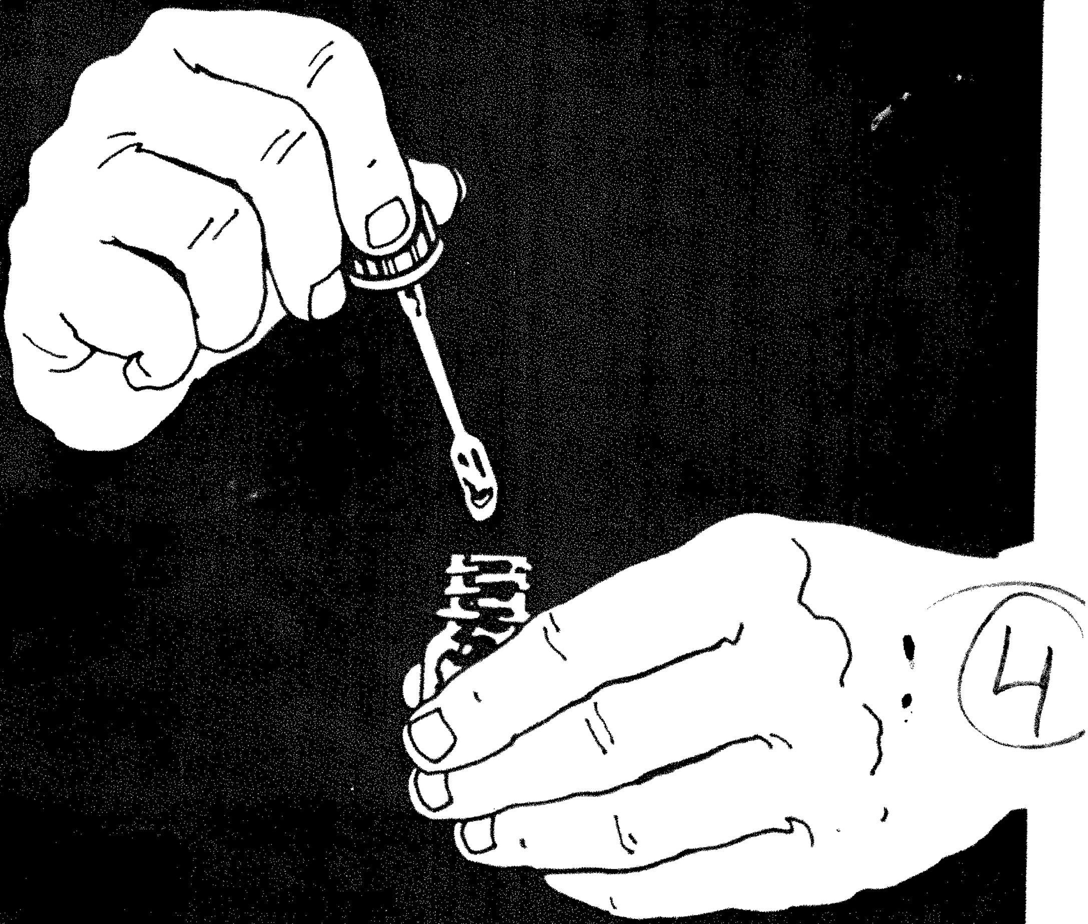

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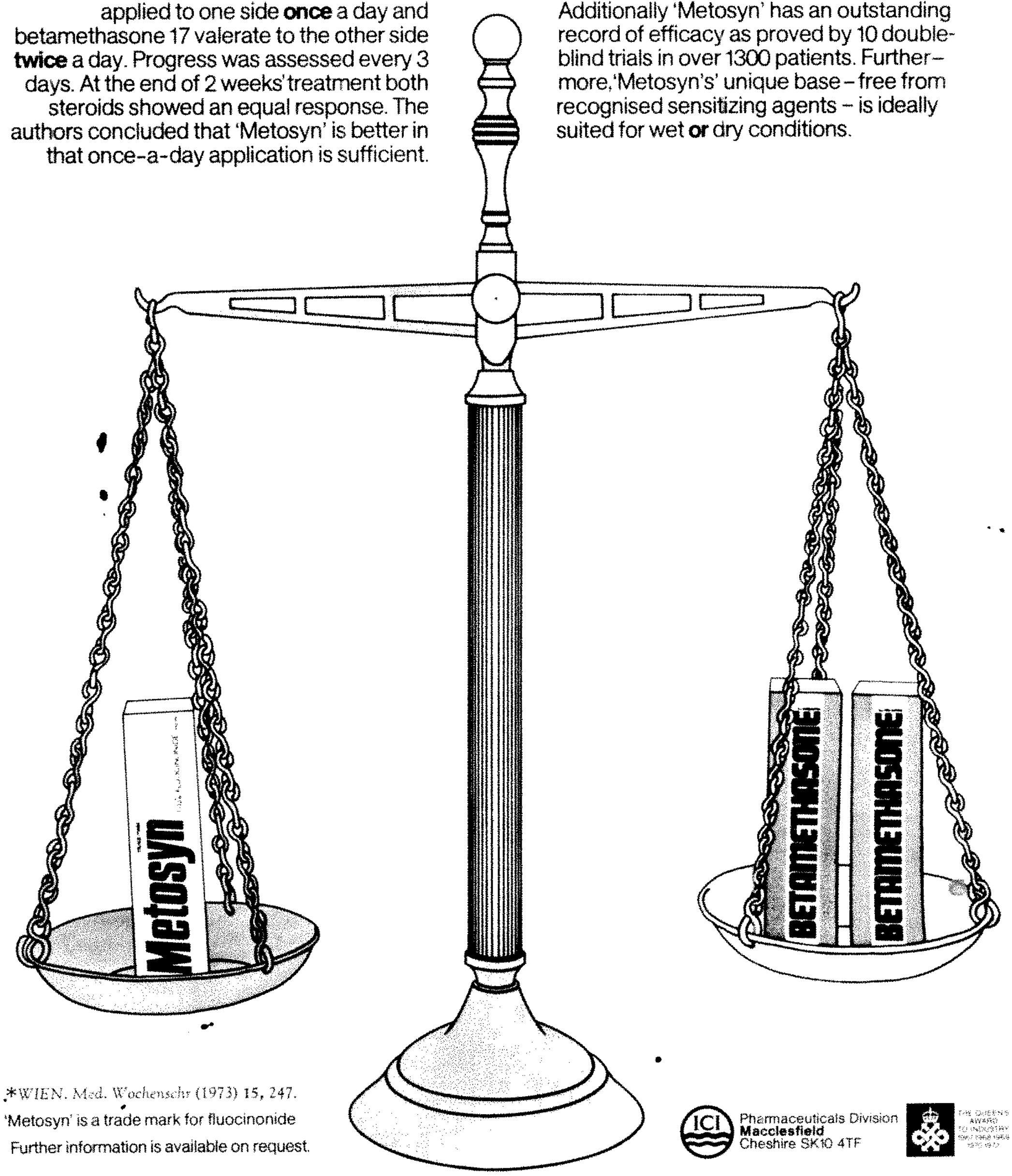

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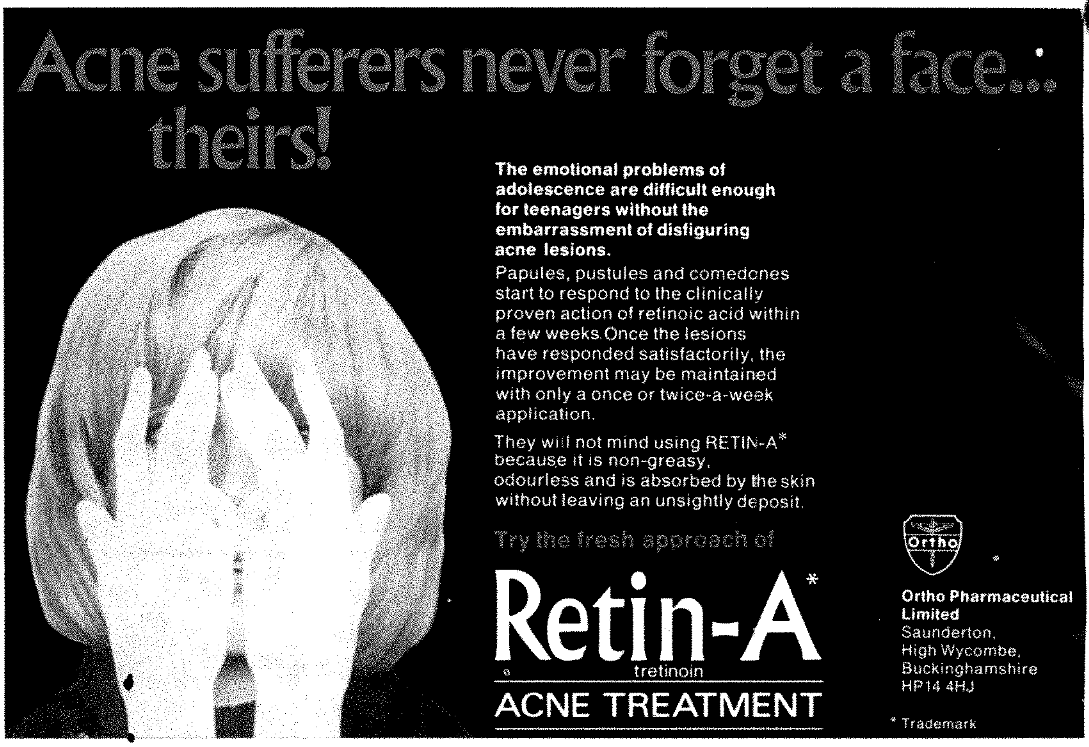

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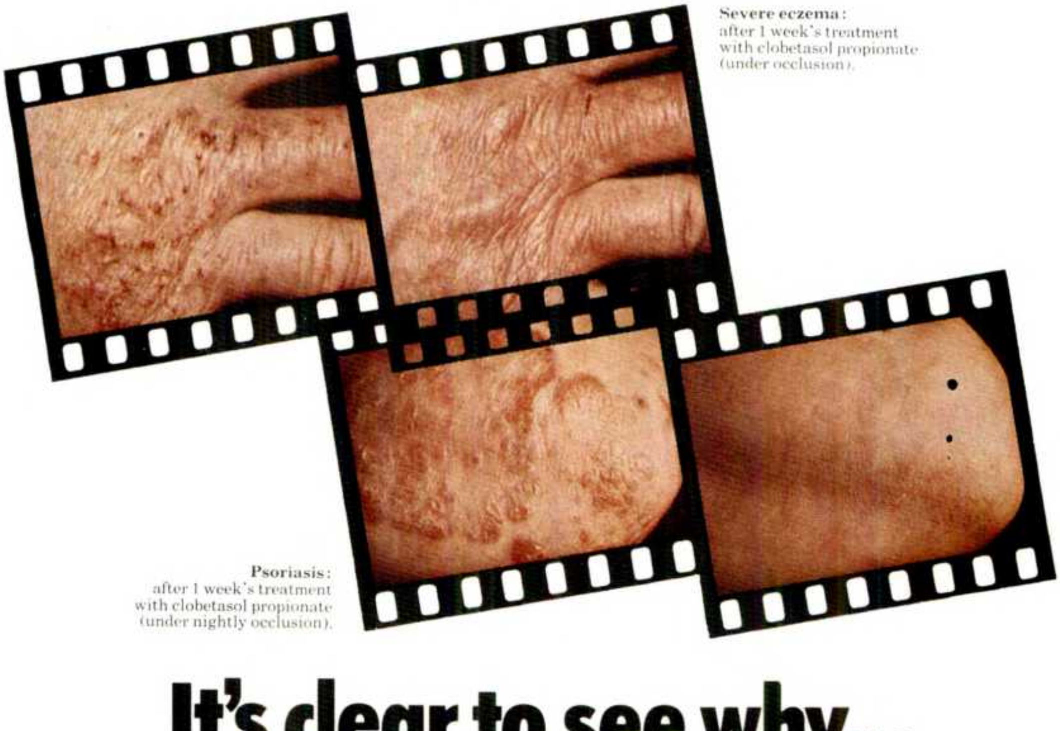

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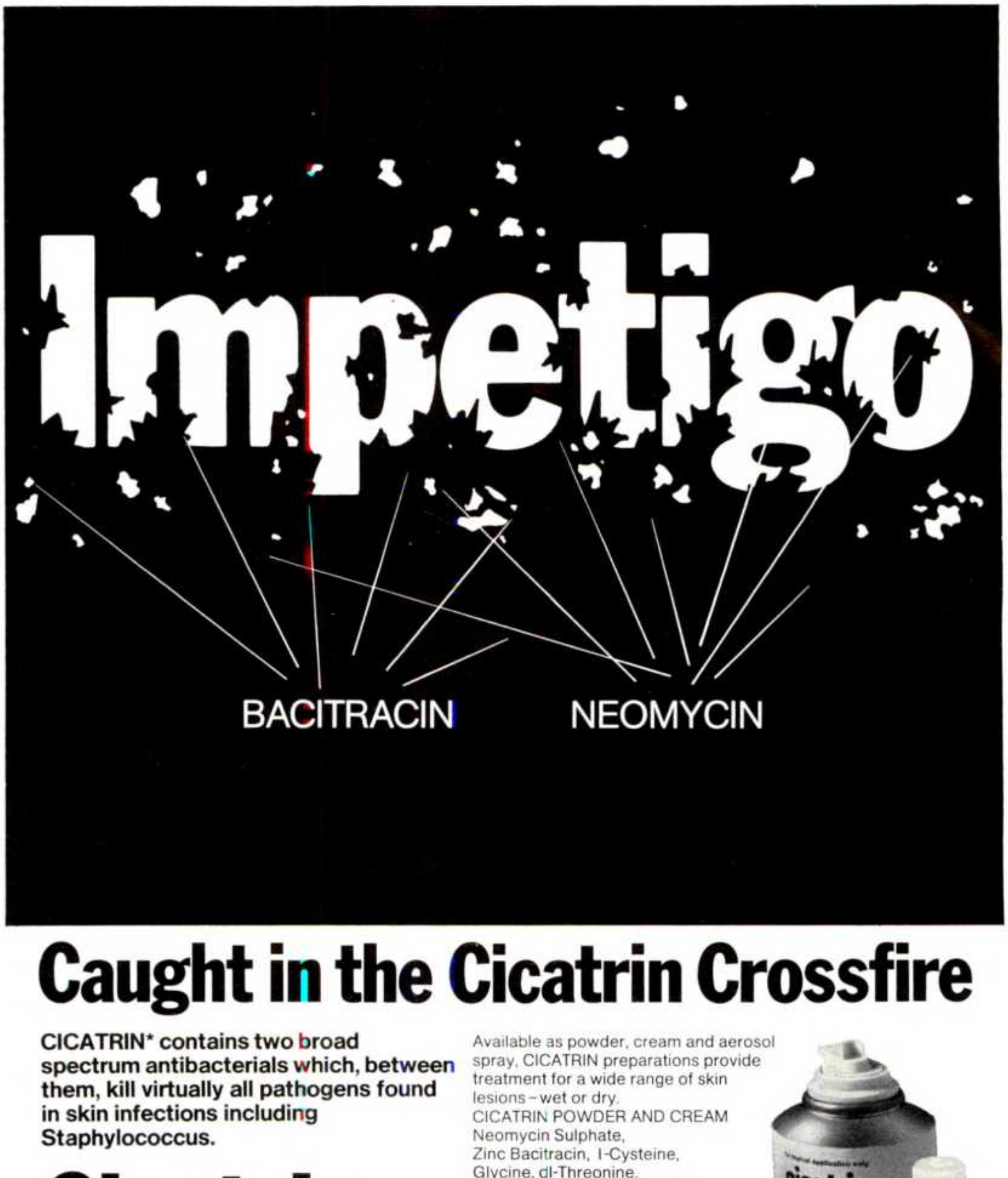

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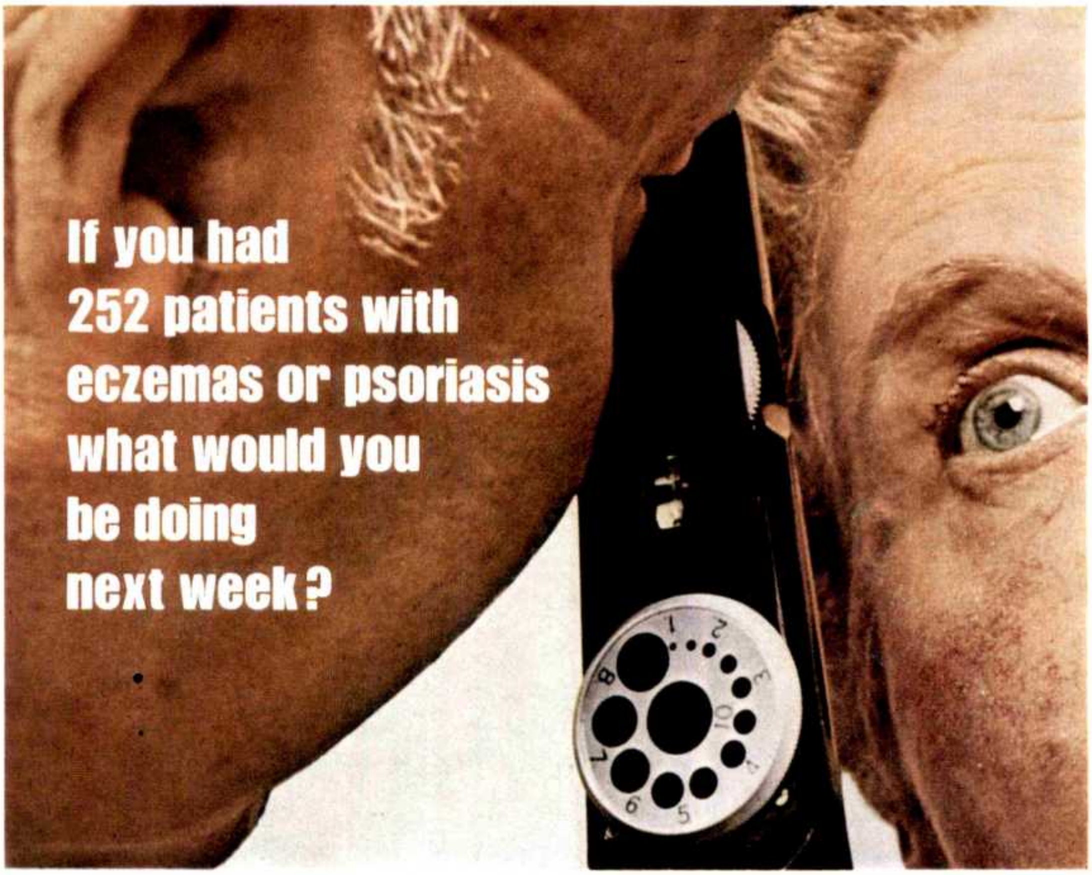

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British Journal of Dermatology (1974) **90**, 245.

Clinical and Laboratory Investigations

A clinical syndrome characteristic of primary Type IV–V hyperlipoproteinaemia

PETER BORRIE AND JOAN SLACK

Skin Department, St Bartholomew's Hospital, and MRC Clinical Genetics Unit, Institute of Child Health, London, W.C.1

Accepted for publication 19 July 1973

SUMMARY

Ten patients with a recognizable clinical syndrome are described. All were men who were overweight and presented with eruptive xanthomata.

All were found to have turbid serum with increased prebetalipoprotein. The post-heparin lipolytic activity was low in the eight patients in which it was measured. Five were diabetic and five had uric acid levels above 7 mg/100 ml.

The presence of chylomicrons was variable and the electrophoretic pattern showed Type IV hyperlipoproteinaemia in some patients and Type V in others. Some patients, while consuming a 'normal Western diet', produced both patterns at different times.

A classification of the hyperlipoproteinaemias which is based on the characteristic abnormalities of lipoprotein distribution as shown by electrophoresis (Frederickson, Levy & Lees, 1967; Beaumont *et al.*, 1970), is now universally accepted. Sometimes, however, there is not complete agreement between the clinical, genetic and biochemical features. This is frequently the case with Types IV and V. For instance, the Type IV electrophoretic pattern may be converted into Type V by minor dietary measures alone, which still leave the patient consuming a 'normal Western diet'; and in families of Type V probands, relations can be found with Type IV pattern (Frederickson & Levy, 1972).

The present communication concerns a group of ten patients, all of whom attended the skin department of St Bartholomew's Hospital on account of cutaneous xanthomata. The clinical pictures and natural histories were remarkably uniform; the biochemical findings, however, varied from patient to patient and also in the same patient from time to time.

CLINICAL FEATURES

Xanthomata

The clinical details are shown in Table 1. All the patients were male, the ages at onset varying between 27 and 47 years in all except one, in whom clinical symptoms presented at the age of 59. Eruptive xanthomata occurred in all the patients and were the sole cutaneous lesion in nine. They varied in number from a single lesion in case 8 to sheets of papules covering large areas of the trunk and limbs in case 7 (Fig. 1). They showed the same propensity as other types of cutaneous xanthomata for being

TABLE 1. The clinical details presented by each patient

Case no.	Sex	Age at onset (years)	Xanthomata	Weight (st	lb)	Height (ft	in)
1	M	40	Eruptive	11	8	5	8
2	M	30	Eruptive	15	2	5	11
3	M	35	Eruptive	14	10	5	10
4	M	27	Eruptive	15	5	6	1½
5	M	31	Eruptive	16	1	5	8
6	M	34	Eruptive	17	3	5	10
7	M	36	Eruptive	13	1	5	8
8	M	47	Eruptive	12	8	5	7
9	M	59	Eruptive Tubero-eruptive	15	8	6	0
10	M	34	Eruptive	13	6	5	10

precipitated by trauma; appearing in one patient in an area of callus on the foot which was regularly abraded with pumice stone, and in another on the beard in which there were large numbers of ingrowing hairs.

In case 9, tubero-eruptive xanthomata were also present on the elbows and knees. This case was also interesting in the fact that eruptive xanthomata were found on the palmar surfaces of the fingers and on the palms. However, they were not confined to the creases and were easily distinguishable from the plane and striate lesions characteristic of Type III (Fredrickson *et al.*, 1967; Borrie, 1969). Indeed, in a total of forty-nine patients referred to the Skin Department on account of the cutaneous symptoms of all types of hyperlipoproteinaemia, we have never seen plane xanthomata of the palms in any other than Type III. No patient had tendon xanthomata or a corneal arcus.

Biopsy of eruptive lesions was perfomed in seven cases. Examination of sections stained for fat under polarized light showed that in every case at least three-quarters of the lipid was neutral fat and only about a quarter was cholesterol. This is the opposite to our findings with the tuberous lesions of Type II and III hyperlipoproteinaemia, in which nearly all the fat is doubly refractile cholesterol. These findings are not quite in keeping with those of Baes, van Gent & Pries (1968), who estimated lipid in xanthomata by chemical methods on excised material. While they found that eruptive xanthomata contain relatively more free fatty acids and triglycerides and less cholesterol than tuberous lesions, they also found that cholesterol deposition in the eruptive xanthomata was still predominant in six out of seven cases of Type IV hyperlipoproteinaemia.

Obesity

All the patients were overweight and all professed large appetites. Remarks such as, 'I could eat all the time', or, 'I'm permanently hungry', were commonplace, while case 2 regularly ate six large meals a day. In some cases they had always been overweight. In others a fairly sudden increase of weight had taken place, often following cessation of regular exercise (discharge from the army; giving up rowing; giving up football). The period between commencing weight gain and appearance of symptoms varied between 4 and 14 years.

Diabetes

A glucose tolerance test was performed in all cases, and five were found to have mild, non-ketotic, maturity-onset diabetes, which was subsequently controlled by diet alone or by diet and oral

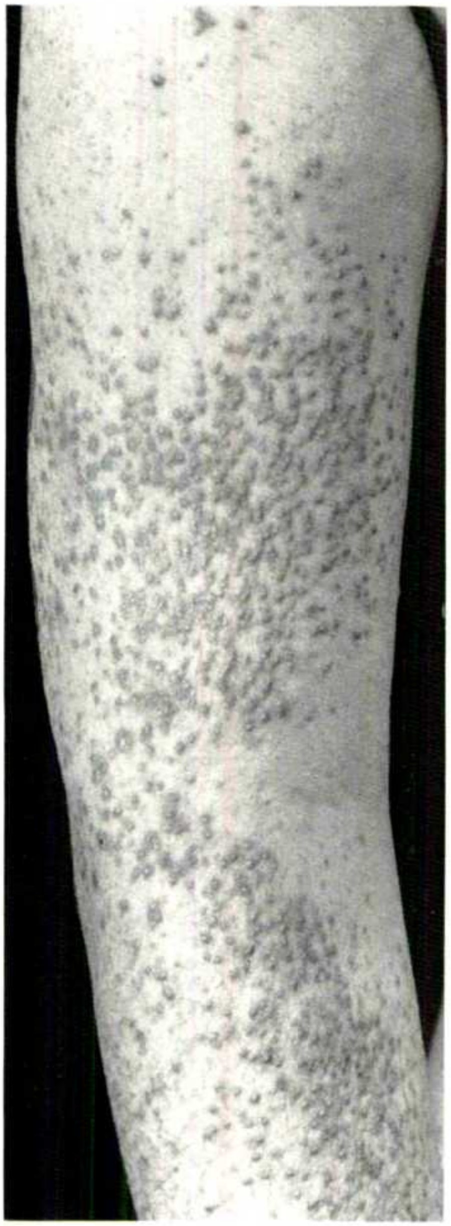

FIGURE 1. Eruptive xanthomata—case 7.

hypoglycaemic agents. Of these five, cases 3 and 5 never exhibited chylomicrons, case 6 produced chylomicrons on some but not all occasions, and cases 7 and 9 were always found to have chylomicrons. No further cases of diabetes have developed during the period of follow-up (Table 2).

Hyperuricaemia

The blood uric acid was investigated and was found to be below 7·0 mg/100 ml in four patients,

TABLE 2. The follow-up period and results of glucose tolerance tests and blood uric acid concentrations in each patient

Case no.	Follow-up (years)	Glucose tolerance test	Uric acid (mg/100 ml)
1	18	Normal	8·6
2	16	Normal	8·0
3	13	Diabetic	8·0
4	10	Normal	9·0
5	8	Diabetic	7·8
6	6	Diabetic	5·2
7	4	Diabetic	7·0
8	2	Normal	6·6
9	1	Diabetic	5·8
10	0·5	Normal	6·9

exactly 7.0 mg/100 ml in one and more than 7.0 mg/100 ml in five (Table 2). Only one patient (case 3) had gout and he was the only one with a family history of gout, his father suffering from it as well.

Abdominal pain

Only three patients suffered abdominal pain. In case 8 it was mild, recurrent, rather ill-defined, but mainly upper abdominal. In case 6 it occurred in attacks of constant epigastric pain lasting for days at a time and on three occasions necessitating hospital admission. However, no cause was found and the symptoms subsided spontaneously. In case 2, recurrent abdominal pain was fairly frequent and culminated one night in an emergency admission with acute symptoms. At laparotomy, acute pancreatitis was found.

Hepato-splenomegaly was present in cases 1, 2, 3 and 5. It slowly subsided with reduction in weight and of plasma lipid levels.

Cardio-vascular disease

All patients were investigated for cardio-vascular disease. There were no symptoms of either cardiac or peripheral vascular disease, and the ECG and peripheral pulses were normal. Case 2 was found to have essential hypertension which responded to routine treatment.

BIOCHEMICAL FEATURES

Biochemical methods

Fasting serum triglycerides were measured by the method of Van Handel & Zilversmit (1957).

Total serum cholesterol was measured by the Sperry–Webb technique (Sperry & Webb, 1950). Measurement of serum cholesterol concentration by this method is not affected by the presence of high triglyceride concentrations.

Electrophoresis of lipoproteins was carried out on paper by the method of Fredrickson *et al.* (1967) and on agarose gel by the method of Noble (1968).

Analytical ultracentrifugation was carried out according to the method of De Lalla & Gofman (1954), in which the low density lipoproteins were isolated by 16 h centrifugation at a solvent density of 1.063 g/ml in the 30.2 rotor of the Spinco Model L ultracentrifuge. The distribution of the lipoproteins was then analysed in a Model E centrifuge, equipped with a phase plate Schlieren diaphragm, in which the rotor temperature was maintained at 26°C. The quantitative distribution was calculated from the Schlieren pattern by transferring its co-ordinates directly on to punched paper tape which was then processed in a suitably programmed digital computer. The programme is designed to produce a schematic print-out which represents the whole lipoprotein distribution as it would be seen 30 min after the start of the run.

Post-heparin lipase was measured by the method of Fredrickson, Ono & Davis (1963).

TABLE 3. The results of lipid examination at the first attendance of the first seven patients

Case no.	Age at time of test (years)	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)	Turbidity
1	40	420	4260	
2	30	643	—	Very heavy
3	36	575	—	Very heavy
4	32	400	—	Very heavy
5	38	440	3700	
6	35	436	—	Very heavy
7	36	660	2300	

TABLE 4. The lipid concentrations and post-heparin lipolytic activity in each patient and in age-matched controls

Case no.	Age (years)	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)	PHLA (μEq/ml/min)
1	58	324	455	0·20
2	48	337	4150	0·19
3	46	335	1990	0·22
4	34	402	1000	0·19
5	41	303	435	0·28
6	40	288	1125	0·22
7	38	271	610	0·16
8	47	760	5240	0·20
9	59	344	1610	—
10	34	512	1500	—
Mean and range in age-matched controls	44·5 (34–59)	220 (173–269)	118 (36–170)	0·41 (0·30–0·48)

Biochemical findings

At the time of the first attendance of the original seven patients, facilities for full biochemical investigation were not available. Those investigations which were performed are shown in Table 3. Triglycerides were not always measured, but the observation of 'heavy turbidity' of the samples implies a notable elevation of serum triglyceride concentration. Subsequent fuller investigations of these patients, together with the other three, are shown in Table 4. It must be noted that when these latter investigations were performed the first seven patients were under treatment, while the last three had not yet commenced treatment. In no case have lipid concentrations become normal.

TABLE 5. The lipoprotein concentrations in mg/100 ml measured by analytical ultracentifugation in six patients and in age-matched controls

Case no.	Age (years)	S_f 0–12	S_f 12–20	S_f 20–100	S_f 100–400
1	58	241	55	323	138
4	34	152	47	460	1430
7	38	202	75	390	378
8	47	0	0	274	1660
9	59	50	0	46	123
10	34	195	0	377	662
Mean and range in age-matched controls	45 (34–59)	301 (206–344)	48 (27–67)	73 (39–151)	22 (3–60)

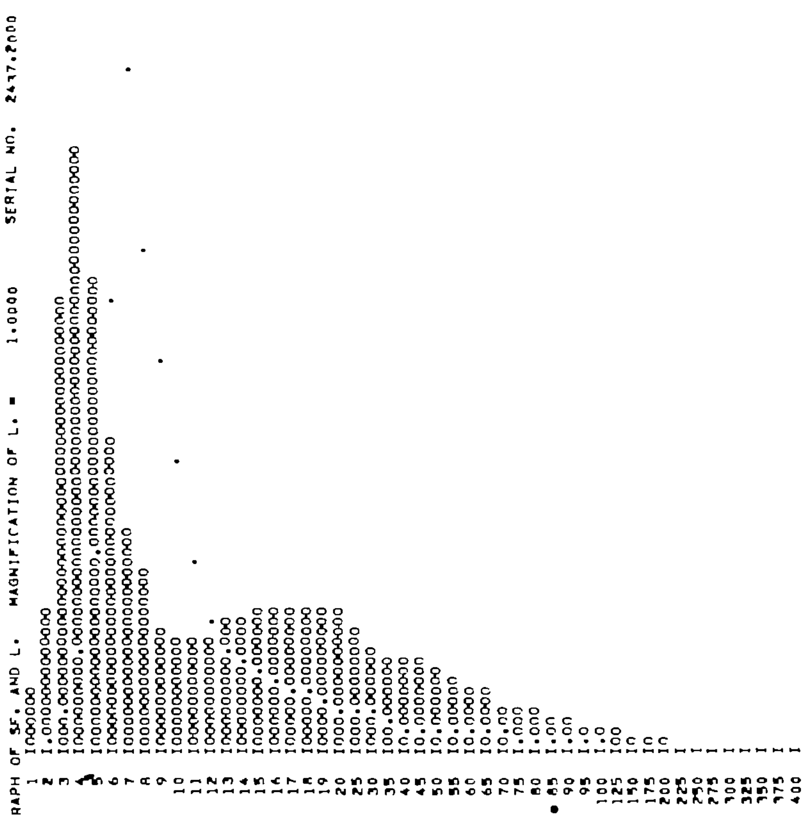

FIGURE 2. Figs. 2 and 3 are schematic printouts from the analytical ultracentrifuge showing change in distribution of low and very low density lipoproteins in case 4 under conditions described in the text. The lipoprotein flotation rate in Svedberg units is displayed on the horizontal axis. The height of each column is proportional to the concentration of lipoprotein at each step along the flotation axis. The expected distribution for a male of 40 years is shown by the dots.

Electrophoresis has been carried out on all patients except case 2, in some on several occasions. In all cases gross elevation of prebetalipoprotein has been observed and this abnormality has persisted at all times. In cases 1, 3 and 5, the presence of chylomicrons has not been demonstrated, and in cases 4 and 6 the presence of chylomicrons has been variable. Case 1 has never been found to have chylomicrons but on one occasion 'floating betalipoprotein' was observed, using both paper and agarose gel for electrophoresis after separation of top and bottom fractions at serum density by preparative ultracentrifugation (Fredrickson *et al.*, 1967). On this occasion the serum sample from case 1 was indistinguishable both on paper electrophoresis and on analytical ultracentrifugation, from Type III hyperlipoproteinaemia. Cases 7, 8, 9 and 10 have so far always exhibited chylomicrons and their electrophoretic patterns have been characteristic of Type V.

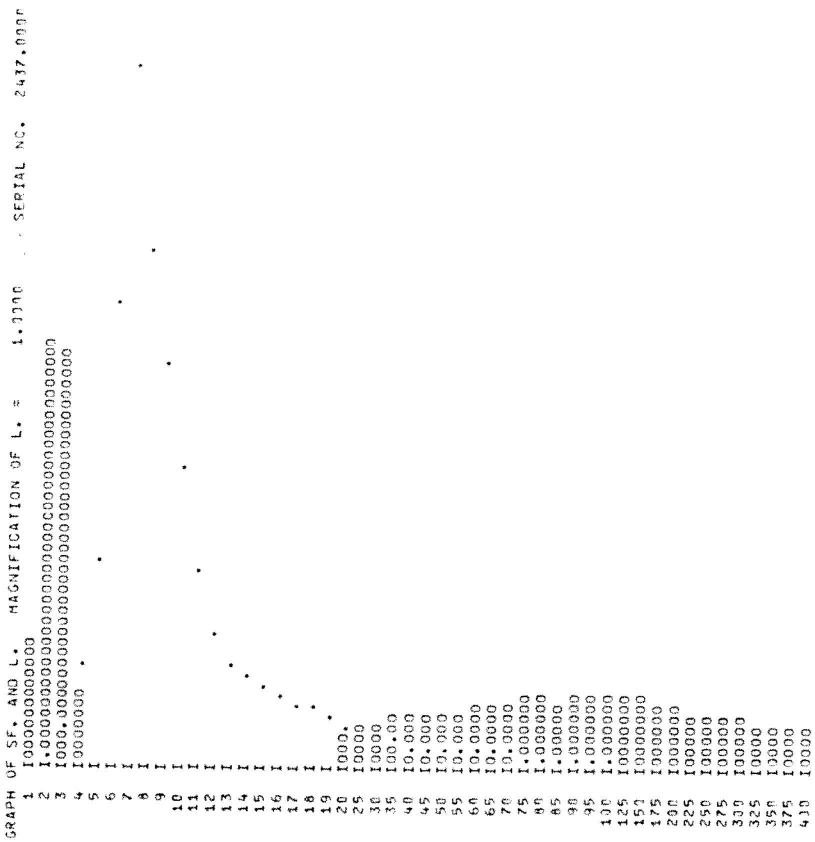

FIGURE 3.

Post-heparin lipase activity has been measured in all patients except cases 9 and 10, and has been found to be consistently low (Table 4).

Table 5 shows lipoprotein concentrations measured by analytical ultracentrifugation. A mean and the range of lipid and lipoprotein concentration in carefully age-matched male controls is also shown for comparison. The investigation was performed on samples from cases 1, 4, 7, 8, 9 and 10. The concentration of lipoproteins of density S_f 0–12 is low, the amount of material at S_f 12–20 was variable and sometimes very markedly reduced, while the material at S_f 100–400 was on all occasions greatly increased. The first pattern obtained from case 4 is shown in Fig. 2 and is characteristic of type IV;

the quantitative measurements on this sample are recorded in Tables 4 and 5. However, with quite small changes in dietary regime (well within the standards of 'a normal Western diet') the pattern changed and became characteristic of Type V hyperlipoproteinaemia and is shown in Fig. 3. At this time the serum cholesterol was 329 mg/100 ml, the fasting triglycerides were 499 mg/100 ml and the analytical ultracentrifuge showed S_f 0–12 was 195; S_f 12–20, 0; S_f 20–100 and S_f 100–400, 662 mg/100 ml. Similar changes have been observed in case 7.

TREATMENT AND COURSE

Treatment consisted of a simple weight reducing diet designed to obtain and maintain weight normal for the patient's height and age; clofibrate in a dose of 1 g twice daily; and oral hypoglycaemic agents where indicated. In all cases the cutaneous lesions resolved in between 3 and 6 months. Recurrences occurred, however, if treatment was not continued. In cases 6 and 7, fresh xanthomata occurred with considerable increase in weight, even though the clofibrate was continued. In cases 1, 3 and 4, on the other hand, recurrences occurred on stopping clofibrate even though the reduction of weight was maintained.

Abdominal pain continues mildly and intermittently in cases 3 and 6, but has never recurred in case 2, in whom an operation for acute pancreatitis was performed.

With vigorous treatment it was possible to get the cholesterol down to normal and the triglyceride to below 500 mg/100 ml. The hyperuricaemia was not affected. However, as the cutaneous lesions disappeared, the patients tended to relax their treatment and their blood lipids rose. As a group they continue, in spite of a slightly raised blood cholesterol and a markedly raised blood triglyceride, to enjoy robust, if slightly over-weight, good health.

DISCUSSION

The clinical features of the present series of patients are remarkably similar to those found by Fredrickson & Levy (1972) in their cases associated with Type V lipoprotein pattern. A comparison of the two series is shown in Table 6. A possible explanation of such differences as there are may lie in the fact that Fredrickson's patients were selected through their electrophoretic pattern, whereas the present series were selected on the characteristics of their cutaneous xanthomata.

TABLE 6. The proportion of patients, expressed as percentages, with Type V hyperlipoproteinaemia experiencing clinical abnormalities in the series of Fredrickson & Levy (1972) compared with the present series

	Abnormal glucose tolerance	Ischaemic heart disease	Peripheral vascular disease	Eruptive xanthoma	Hepatosplenomegaly	Abdominal pain	Pancreatitis	Hyperuricaemia
Fredrickson & Levy (1972)	82	4·5	0	45	32	75	41	41
Present series	55	0	0	100	44	33	10	55

The most consistent biochemical findings in these patients have been a turbid serum with elevation of triglyceride concentrations and an intense prebetalipoprotein band demonstrable on electrophoresis. These characteristics have never disappeared, even when treatment has been maximal and all eruptive xanthomata have vanished.

Although post-heparin lipolytic activity has not been measured on more than one occasion the levels of lipoprotein lipase in the eight patients examined were consistently low.

Analytical ultracentrifugation in the six patients examined showed a characteristic pattern with low concentrations of material at S_f 0–12 and grossly elevated concentrations of lipoproteins in the S_f 100–400 range. It seems possible that it is this pattern on analytical ultracentrifugation that distinguishes the patients in this group most clearly from other patients with Fredrickson's Type IV hyperlipoproteinaemia who do not exhibit the clinical syndrome described in our ten patients.

Some of the time these patients, while consuming a diet of 'normal Western standards', have exhibited electrophoretic characteristics of Type IV hyperlipoproteinaemia but, perhaps because of the additional defect of reduced lipoprotein lipase activity, they are more prone than others who have normal lipase activity, to demonstrate chylomicrons during periods of natural dietary exuberance, thereby converting their electrophoretic pattern into Type V. Thus it is clear that repeated biochemical investigation performed on a group of patients such as the present series, who exhibit consistently similar clinical features, may not reveal similarly consistent biochemical findings and in particular the distribution of their lipoproteins on electrophoresis may be very variable.

ACKNOWLEDGMENTS

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The association of familial hyperbetalipoproteinaemia with Waardenburg's syndrome

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SUMMARY

Six members of a family were discovered to have many of the manifestations of Waardenburg's syndrome, and increased levels of total serum lipids. The latter was marked in most of the members, especially in those who also had xanthomata.

In this study the clinical and laboratory findings are presented. Relationships between these syndromes, both of which are known to be autosomal dominant traits, are examined.

Waardenburg, in 1951, described a syndrome with the following components: white forelock; unilateral or bilateral congenital deafness; lateral displacement of the inner canthi and the inferior lacrimal puncti; high broad nasal root; some degree of heterochromia of the irides; and confluence of the eyebrows.

From his study of sixteen pedigrees, Waardenburg concluded that the syndrome has an autosomal dominant inheritance with variable penetrance of the individual components. Since Waardenburg's initial description, few variants of the syndrome have been described.

Keizer (1952) described Waardenburg's syndrome (WS) in a Dutch family. Wildervanck (1957) reported five Dutch cases. McKenzie (1958) reported the occurrence of WS in six individuals in one family. Partington (1969) described an English family of which five members in four generations showed various components of the syndrome. In the same year, Fisch (1957) reported the first histological examination of the inner ear in WS. Di George, Olmstead & Harley (1960) were the first to report the occurrence of WS in negroes; they demonstrated pigmentary changes in the fundus of the eye.

Familial hyperbetalipoproteinaemia (FHBL) is inherited as an autosomal dominant trait (Alvord, 1949; Leonard, 1956; Nevin & Slack, 1968; Slack & Borrie, 1971).

Clinically the most important feature is the deposition of lipid in the tissues, some patients showing tuberous xanthomata, others developing vascular complications in early life; in homozygotes xanthomata may be present at birth (Khachadurian, 1964). Arcus corneae is present in 10% of cases under 30 years of age and in about 50% of cases above this age (Gofman *et al.*, 1954a, b).

CASE REPORTS

Six members of one family are described. The father (case 1) and the mother, his first wife (case 2),

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are double first cousins. They have three children, two boys (cases 3 and 4) and a girl (case 5). The youngest member of the family (case 6) is a girl whose mother is the second wife. This second wife was not related to the father; she was divorced and she left Kuwait. There were no other children of these two marriages.

Case 1

Male, 39-year-old, Iraqi, is a workman in the Ministry of Education. He had no complaint and was

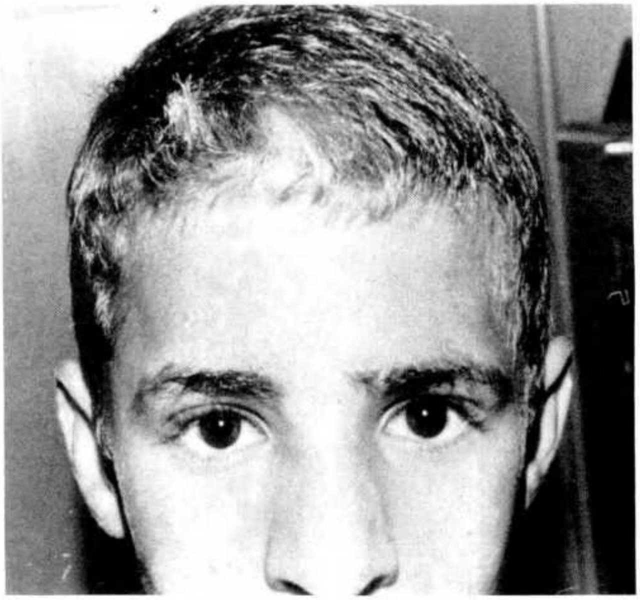

FIGURE 1. Case 4. White forelock. Lateral displacement of inner canthi and of inferior lacrimal puncti. High broad nasal root. Confluence of eyebrows with hypertrichosis of their medial ends. Premature greying of hairs.

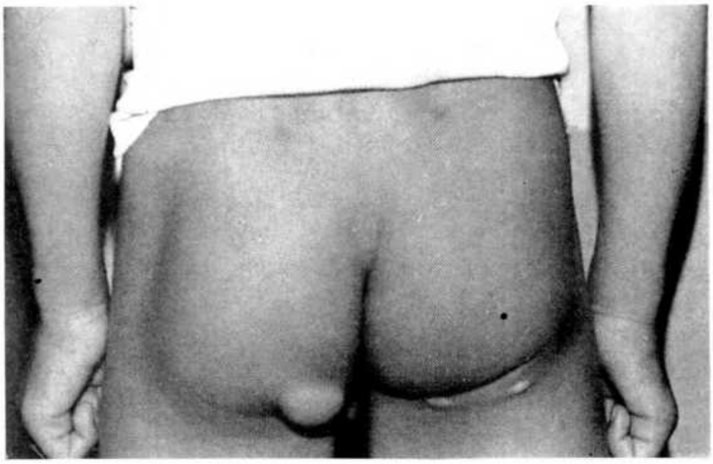

FIGURE 2. Case 4. Xanthoma tuberosum. Depigmented patches.

asked to attend the clinic after the examination of his son (case 3) in April 1972 in the dermatology clinic of the School Health Centre.

On examination, the clinical findings of importance were: white forelock; confluence of eyebrows; a leukoderma over the right knee and greying of the hairs on the anterior and medial aspects of his right leg. Eyes: visual acuity 6/9 both eyes; no heterochromia; arcus corneae in both eyes; three pigmented naevi on right iris. Ears: clinically normal, audiogram normal. ECG: no abnormality detected. X-ray chest: no abnormality detected.

Laboratory investigations (see Table 1).

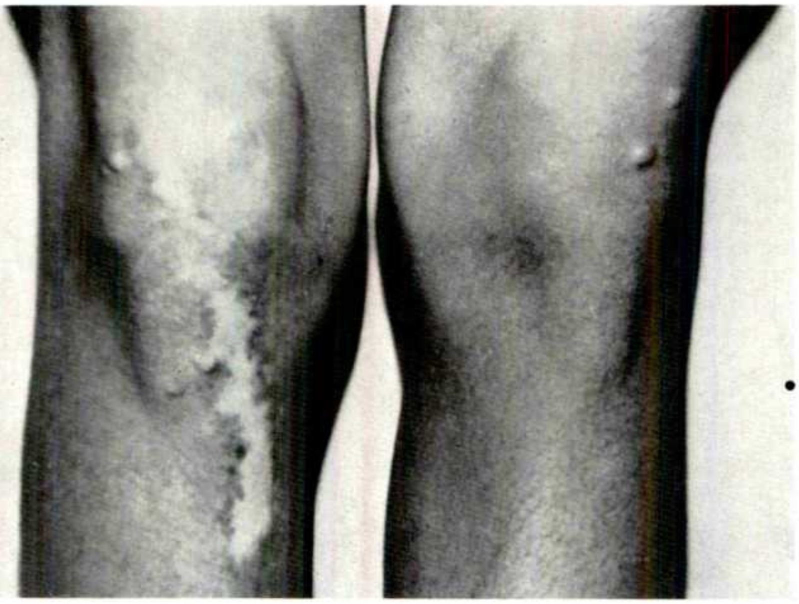

FIGURE 3. Case 4. Xanthoma tuberosum.

Case 2

Female, 35-year-old, the first wife of case 1, housewife, married for 19 years. She has three children, two boys and one girl. No history of abortion, premature labour or still births was obtained. She had no complaints. She was asked to attend the clinic after the examination of her son (case 3).

On examination, the clinical findings of importance were: white forelock; lateral displacement of the inner canthi and inferior lacrimal puncti; confluence of eyebrows. Eyes: heterochromia of irides; visual acuity, right 6/24, left 6/18; bilateral persistent very fine and floating pupillary membrane.

Laboratory investigations (see Table 1).

Case 3

Male, 15-year-old, the son of his father's first wife, is an intermediate school student. This was the index case for the family. He was referred by the school doctor to our clinic 7 years ago with xanthomata. On examination were found: white forelock; lateral displacement of the inner canthi and of the inferior lacrimal puncta; high broad nasal root; confluence of eyebrows; some degree of heterochromia of the irides; premature greying of the hair; xanthomata of tuberous type on the inner side of the right gluteal fold; aortic stenosis. Ears, clinically and audiometrically normal. X-ray chest: left ventricular enlargement. ECG: characteristic of left ventricular preponderance.

On direct questioning, the patient stated that he felt pain in the chest after moderate exercise.

Laboratory investigations (see Table 1).

Case 4

Male, 13-year-old, the second son of his father's first wife, is an intermediate school student. The positive findings were: white forelock; lateral displacement of the inner canthi and the inferior lacrimal puncti (Fig. 1); high broad nasal root (Fig. 1); confluence of eyebrows (Fig. 1); premature greying of the hair (Fig. 1); xanthomata of 5 years' duration on the elbows, knees (Fig. 2), the anterior aspect of the right thigh (Fig. 2), and the gluteal region (Fig. 3). Patches of leukoderma on the right knee, the anterior aspect of the right thigh (Fig. 2) and the right upper abdominal wall. Eyes: visual acuity 6/6 on both sides; persistent very fine pupillary membrane in right eye. Ears: clinically and audiometrically normal. ECG and X-ray chest: no abnormality detected.

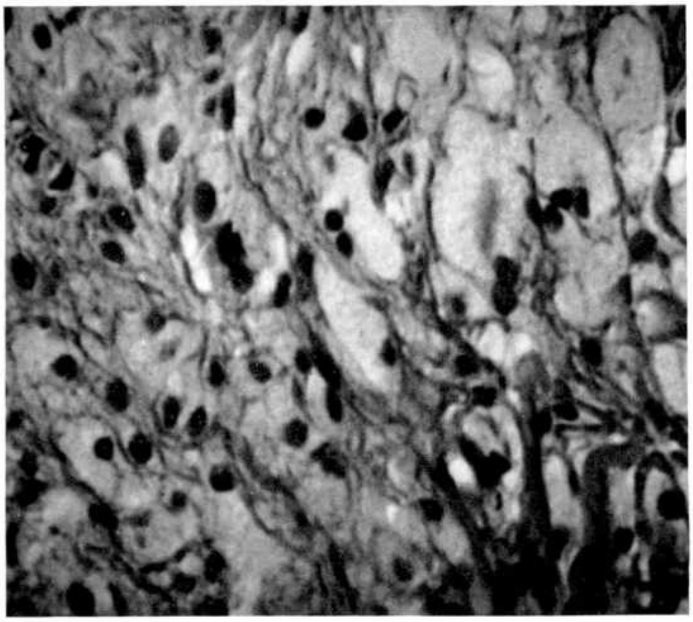

FIGURE 4. Xanthoma cells in the dermis with slight fibrosis (× 400).

Laboratory investigations (see Table 1).

A punch biopsy was taken from the xanthomata over the anterior aspect of the right thigh. The xanthoma selected was that nearest to the depigmented patch (Fig. 2). The histopathological findings (Fig. 4) were: aggregates of foam cells (xanthoma cells) among fibrous tissue occupy the dermis. Both types of xanthoma cells, mononuclear and multinuclear (Touton giant cells), are evident. The appearance is that of xanthoma tuberosum.

Case 5

Female, 11-year-old, the daughter of the first marriage, a primary school student with the following positive findings: white forelock; lateral displacement of the inner canthi and of the inferior lacrimal puncta; high broad nasal root; confluence of eyebrows; premature greying of the head hair; widespread leukoderma, mainly over the forehead, the anterior abdominal wall and the flexor and extensor aspects of both legs (Fig. 5). Eyes: visual acuity 6/6 on both sides; pigmentary deposit on the lens of the left eye denoting mild healed uveitis; persistent pupillary membrane in the right eye. Ears: clinically and audiometrically showed bilateral congenital deafness. ECG and X-ray chest showed no abnormality.

Laboratory investigations (see Table 1).

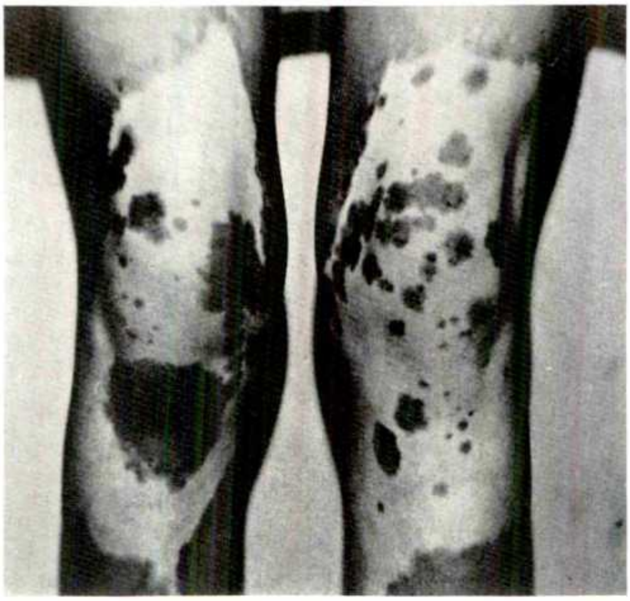

FIGURE 5. Case 5. Depigmentary changes.

Case 6

Female, 10-year-old, the child of the father's second wife; she showed the following: white forelock; lateral displacement of the inner canthi and of the inferior lacrimal puncta; high broad nasal root; hypertrichosis of the medial ends of the eyebrows. Eyes: visual acuity 6/6 both eyes; persistent fine floating pupillary membrane in both eyes. Ears: clinically and audiometrically normal. ECG and X-ray chest normal.

Laboratory findings (see Table 1).

Further investigations

In addition to the investigations reported in the case histories and in Table 1, all patients were thoroughly examined and the following investigations were carried out with uniformly normal results: complete haematological examination; routine examination of urine and stools; liver function tests; kidney function tests; VDRL; protein-bound iodine; antistreptolysin O titre; SGOT. In addition, cardiac catheterization was considered necessary for case 3.

The total serum lipids were estimated by the method of Fringes & Dunn (1970), serum cholesterol by the method of Zlatkis, Zak & Boyle (1953), and serum triglycerides by the methods of Eggestein & Kreutz (1966). Lipoprotein electrophoresis was performed on cellulose acetate membrane after Chin

& Blankenhorn (1968) and on agarose after Noble (1968). The patterns were typed according to Fredrickson & Lees (1967).

The biochemical investigations were repeated six times at about monthly intervals. The figures reported in Table 1 are the mean values of the six results.

TABLE 1. Biochemical investigations

Case no.	Age (years)	Sex	Serum appearance	Total lipid (mg/100 ml)	Total cholesterol (mg/100 ml)	Serum triglyceride (mg/100 ml)	Serum uric acid (mg/100 ml)	Fasting blood sugar (mg/100 ml)	Electrophoretic findings
1	39	M	Clear	1325	462	252	6·3	91	Increased density and thickness of beta and prebeta bands. Fredrickson's type IIB
2	35	F	Clear	843	462	86	4·5	95	Moderate increase in density and thickness of beta band. Fredrickson's type II
3	15	M	Clear	1428	581	106	7·3	90	Increased density and thickness of beta band. Fredrickson's type II
4	13	M	Clear	1652	686	92	6·3	109	Increased density and thickness of beta band. Fredrickson's type II
5	11	F	Clear	929	260	95	4·3	113	Slight increase in density and thickness of beta band. Fredrickson's type II
6	10	F	Clear	826	280	102	5·2	98	Slight increase in density and thickness of beta band. Fredrickson's type II

DISCUSSION

Both FHBL and WS are considered to be genetic abnormalities that can be transmitted as autosomal dominant traits.

In WS, this genetic abnormality has been attributed to certain modifications in early embryonic life of the blood supply of the first visceral arch. These modifications could result from an anomaly of the stapedial artery (Campbell, Campbell & Swift, 1962). The association of hyperlipaemia with WS has not been reported before. This association could be explained by either of two possibilities. The first is that by chance the two traits were segregating both from the father and his first wife, and that they are independent. The second explanation, which we favour, assumes that both traits represent manifestations of the same gene mutation.

In the six cases reported in this study, it would appear that the higher the level of lipid in the serum the more marked are the manifestations of WS. This applies also to the betalipoproteins, i.e. the higher their level in the serum the greater the number of the components of WS which are manifest. This is evident particularly in cases 3 and 4. In contrast, cases 5 and 6 have lower levels of lipids and betalipoproteins in their sera and show less severe manifestations of WS. They have no xanthomatous lesions.

FHBL is inherited as an autosomal dominant trait (Slack & Borrie, 1971). In the heterozygote the presence of type II hyperlipoproteinaemia or of tendon xanthomata is a major criterion (Fredrickson, Levy & Lees, 1967; Fredrickson & Levy, 1972). On the other hand, homozygotes show the type II pattern with an increased LDL concentration (about twice that in the heterozygote of the same kindereds) and both parents must be either heterozygotes or homozygotes for type II hyperlipoproteinaemia. Moreover, the skin lesions in homozygotes may appear very early in childhood, and such individuals may also develop ischaemic heart disease or aortic stenosis early in life (Slack & Borrie, 1971). The genotypes of cases 3, 4 and 5 seem to be homozygotes, because: both parents have increased serum cholesterol levels and the electrophoretic pattern of hyperbetalipoproteinaemia; xanthomata appeared at an early age in cases 3 and 4; a vascular lesion, namely aortic valvular stenosis was detected in case 3, before the age of 20 years.

In the cases considered as homozygous hyperlipoproteinaemics, the manifestations of WS seem to be prominent, and nearly complete. In these three cases WS may also have been inherited in a homozygous manner, since it is present in both parents. This explains why we favour the second hypothesis mentioned above. If indeed FHBL and WS represent the expression of a single gene why have the case previously reported as WS not shown FHBL? There are a number of possible explanations.

(a) Recorded cases of WS are very few, although WS is not as uncommon as this sparsity of literature suggests. This low frequency is an obstacle to evaluation of the precise nature of the syndrome and to determining the underlying biochemical background.

(b) In the family reported, cases 1 and 2 show manifestations of WS without any sign or symptom suggesting the diagnosis of the hyperbetalipoproteinaemia. Their high serum lipid level was discovered only after investigation of their children with cutaneous xanthomata. The serum lipid levels of the whole family were then estimated and proved to be high in most members.This would not have been discovered if cases 1 and 2 had been the presenting cases. Thus, associated FHBL may have been overlooked in some cases of WS.

(c) The authors cannot trace any study of serum lipid levels or of the electrophoretic pattern of serum lipoproteins in cases of WS in the literature. This may indicate either that the serum levels of lipids and lipoproteins in these cases were not elevated or that they were higher than normal but not associated with any cutaneous or visceral manifestations which would have led to the investigation of the lipid levels. The absence of such manifestations is not uncommon in cases of familial hyperlipaemia (Aldersberg, 1955).

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The response of the sweat glands to β-adrenergic stimulation with isoprenaline

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SUMMARY

Sweat responses were obtained in fifteen out of eighteen subjects, using local injections of different concentrations of isoprenaline. In the majority of these subjects injections of 10^{-3} mg in 0·1 ml were the most effective.

The sweat response could not be re-induced at the same site.

No sweat responses could be obtained from sites which had been previously infiltrated with an equivalent concentration of propranolol (Inderal, ICI).

The possible mechanisms of the sweat response and whether these responses are analogous to those observed in the submaxillary glands of animals are discussed.

In preliminary experiments it was observed that intracutaneous injections of 10^{-3} mg isoprenaline in 0·1 ml normal saline elicited sweat responses in eight out of thirteen subjects (Warndorff, 1972a). The curves obtained resembled those seen in spontaneous sweat secretion, and the responses could be terminated by atropine or scopolamine. The responses were therefore clearly different from those induced by adrenaline or noradrenaline (Foster, Kinsbury & Weiner, 1970; Warndorff & Neefs, 1971).

In the previous report, it was discussed whether sweat responses to isoprenaline could imply the presence in the sweat glands of β-receptors, as well as α-receptors to which the responses to (nor)-adrenaline have been attributed (Haimovici, 1950; Rothman, 1954).

The conditions in the eccrine sweat glands could be analogous to those in other cholinergic exocrine glands such as the submaxillary glands of the dog and rat, in which the presence of α- as well as of β-receptors has been postulated (Yoshida *et al.*, 1967; Emmelin, Ohlin & Thulin, 1969). Their significance is still obscure.

From the evolutionary point of view, the eccrine sweat glands may have developed from adrenergic apocrine sweat glands (Kuno, 1956; Ikai *et al.*, 1970). The presumably adrenergic apocrine sweat glands in the hairy surface of the dog are known to respond to both adrenaline and acetylcholine (Aoki, 1955). Recently they have been shown to respond to isoprenaline as well (Cotton & van Hasselt, 1973).

Further studies concerning the effect of isoprenaline on the human eccrine sweat glands were considered necessary. This paper deals with experiments regarding the most effective concentration of isoprenaline, the effect of repeated injections and the effect of the β-blocking agent propranolol (Inderal, ICI) on the responses.

MATERIALS AND METHODS

The subjects were seven healthy volunteers and sixteen dermatological out-patients with various dermatoses. Their ages ranged between 15 and 49 years.

The investigations were carried out between April and November 1972, on the volar aspect of the forearm, at room temperatures between 21 and 24°C. The subjects were comfortably seated, relaxed, and adapted to the environment. Sweat gland activity was measured by means of the electrolytic water analyzer ('Meeco') and a ventilated cup, as described by van Gasselt & Vierhout (1963).

For each set of tests the dilutions of isoprenaline and of propranolol were freshly prepared in normal saline. Approximately 0·1 ml of the diluted solutions was injected.

Experiments

(1) *The most effective concentration of isoprenaline.* In eighteen subjects (see Table 1) the sweat glands at different sites on the volar aspect of the forearm were stimulated with different concentrations of isoprenaline. The distance between the sites was at least 4 cm. The concentrations used varied from 10^{-7} to 10^{-4} g/ml (10^{-5} to 10^{-2} mg per 0·1 ml).

The sweat responses were recorded and the maxima of the responses were compared.

(2) *The effect of repeated injections of isoprenaline.* In six subjects (three male volunteers, three male patients) who showed good sweat responses to 10^{-3} mg isoprenaline, the injection was repeated at the moment when the response to the first injection had come to an end. Both injections were given via an indwelling needle which was connected with a tuberculin syringe by a plastic tube. In four of the subjects the test was carried out twice at different sites. So the total number of tests was ten.

(3) *The effect of the β-blocking agent propranolol on the responses.* In six out of the eighteen subjects from experiment 1 (see above), the injection of isoprenaline which had elicited the strongest response was repeated at another site, this site having been infiltrated with an equivalent concentration of propranolol 10 min before. Isoprenaline and propranolol have about the same molecular weight. Equivalent concentrations by weight may therefore be considered to be approximately equimolar.

In a seventh subject who showed very marked responses to isoprenaline, several tests were carried out with different concentrations of propranolol (10^{-5} to 10^{-3} mg per 0·1 ml).

RESULTS

The character of the response curves, the length of the delay-times between the injection and the onset of the responses, and the duration of the responses were in general similar to those observed in the preliminary investigations (Fig. 1).

Table 1 summarizes the effects of the different concentrations of isoprenaline in the eighteen subjects.

The effect of repeated injections of isoprenaline

Only in one test was the second injection also followed by a sweat response, and then it was notably smaller than the first response. In the other nine tests, there was no sweat response to the second injection (see Fig. 1).

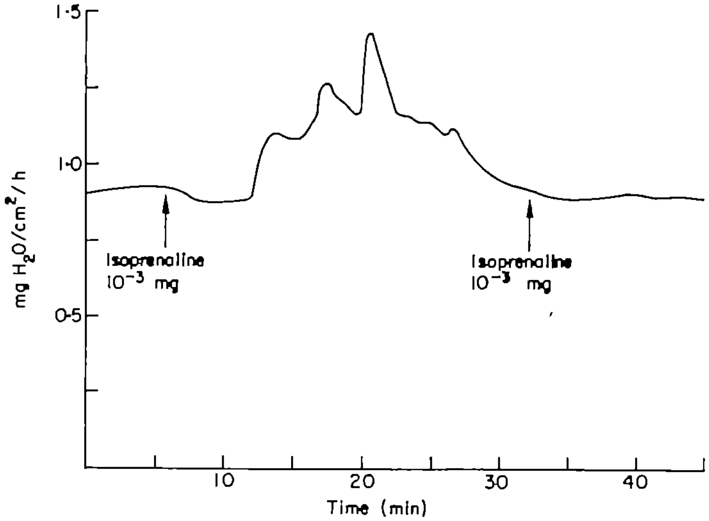

FIGURE 1. The response of the sweat glands to two successive injections of isoprenaline at the same site.

TABLE 1. The responses of the sweat glands to intracutaneous injections of different concentrations of isoprenaline at different sites of the volar aspect of the forearm in eighteen subjects. Values are expressed in mg water per cm^2 per h

Subject	Sex	Diagnosis	IP*	10^{-5} mg	10^{-4} mg	10^{-3} mg	10^{-2} mg
1	F	Healthy	0·50	—	0	1·20	0
2	M	Healthy	0·64	0	0	0	0
3	M	Healthy	0·86	—	0	0	0
4	M	Healthy	0·46	—	0	0	0
5	M	Healthy	0·78	0	3·20	0	0
6	M	Pruritus/asthma	0·80	0	7·50	15·80	8·40
7	F	Neurodermatitis	0·60	0	1·20	6·18	1·48
8	F	Neurodermatitis	0·52	—	0	1·54	0
9	F	Chronic urticaria/asthma	0·64	—	1·04	1·16	3·60
10	M	Psoriasis/neurodermatitis	0·70	—	0	6·42	4·92
11	F	Psoriasis	0·62	—	1·26	2·16	1·26
12	M	Psoriasis	0·52	—	0	1·18	1·34
13	M	Lichen simplex	0·56	—	0	0	1·04
14	F	Contact eczema	0·58	0	0·92	0·96	0
15	F	Acne vulgaris	0·82	—	0	0	4·30
16	F	Pruritus	0·56	—	0	1·58	0
17	M	Trichophytosis	0·68	0	5·52	3·12	—
18	M	Hyperhidrosis gen.	0·58	2·10	16·0	3·60	3·80

* IP, insensible perspiration.

The effect of propranolol on the sweat response

In none of the seven subjects did sweat responses to isoprenaline occur in sites which had been previously infiltrated with an equivalent concentration of propranolol. In the subject who showed very marked responses to different concentrations of isoprenaline it was observed that smaller than equivalent concentrations of propranolol inhibited the response to isoprenaline but did not abolish it.

DISCUSSION

From Table 1 it can be seen that the sweat glands responded to one or more of the different concentrations of isoprenaline in fifteen out of the eighteen subjects. In eight of these fifteen subjects the response was maximal to 10^{-3} mg, in four to 10^{-2} mg and in three to 10^{-4} mg. Only a small response was obtained in one of the seven subjects tested with 10^{-5} mg, although five of these subjects showed fair to strong responses to higher concentrations.

The data suggest that the effective concentrations of isoprenaline are in the same range as those of (nor)adrenaline (Wada, 1950; Chalmers & Keele, 1951, 1952; Foster *et al.*, 1970; Warndorff & Neefs, 1971).

From Table 1 it appears that injections of concentrations higher than that maximally effective did not elicit equivalent, but actually smaller responses. This might be attributed to local differences in the sensitivity of the sweat glands. Cotton & van Hasselt (1972), who observed the same phenomenon in quantitative measurements of the sweat responses to catecholamines in dogs, suggested an inhibitory action of supra-maximal concentrations.

The findings that twelve of the eighteen subjects responded to injections of 10^{-3} mg isoprenaline and that good to strong responses were usually obtained from atopic subjects (asthma, atopic dermatitis) were in good agreement with our previous observations. In atopic subjects the sensitivity of the sweat glands to isoprenaline seems to run parallel to that to acetylcholine (Warndorff, 1970, 1972b). The same seems to apply to patients suffering from generalized hyperhidrosis.

The sweat responses to isoprenaline were generally not repeatable at the same site. 'Non-repeatability' is a known feature in the response of the sweat glands to injections of (nor)adrenaline (Collins, Sargent & Weiner, 1959; Foster *et al.*, 1970; Warndorff, 1972b). This phenomenon has been attributed to the vasoconstrictive action of (nor)adrenaline (Foster *et al.*, 1970). This is in itself an unsatisfactory explanation (considering the co-existence of strong vasoconstriction and profuse sweat secretion in shock and in crises caused by a phaeochromocytoma) and surely cannot hold true for isoprenaline which induces slight vasodilatation.

In normal subjects, sweat glands stimulated with isoprenaline show their normal or even increased responses to cholinergic agents (Gibson, 1968; Warndorff, unpublished observations); increased responses have been observed particularly in cystic fibrosis patients (Gibson, 1968). These data imply that the refractory state cannot be attributed to an inhibitory action of isoprenaline on the secretory cells.

Sweat responses to isoprenaline could not be obtained from sites which had previously been infiltrated with equivalent concentrations of propranolol; lower concentrations seemed to inhibit the responses. Hemels (1970) observed that local injections of propranolol increased the sensitivity of the sweat glands to minimal quantities of acetylcholine in normal subjects, while they did not affect the already increased sensitivity in atopic subjects. The blocking effect of propranolol on the sweat responses to isoprenaline is therefore not likely to depend upon inhibition of the secretory cells. Propranolol is considered to be a rather specific blocking agent of β-receptors (Ariens, 1970).

The results of these investigations could support the idea that the sweat responses to isoprenaline depend upon the presence of β-receptors in the sweat glands.

In the submaxillary glands of the dog and of the rat, the existence of α- and of β-receptors has been postulated because of the quantitative and qualitative (including chemical) differences between adrenergic (α and β) and cholinergic secretion (Yoshida *et al.*, 1967; Emmelin *et al.*, 1969). The same arguments could apply to the eccrine sweat glands as far as differences in the character of the responses and in the amounts of sweat produced are concerned (Warndorff & Neefs, 1971; Warndorff, 1972b). The hypothesis needs support from chemical analyses of adrenergic (α and β) sweat. These, however, are made difficult because adrenergic sweat responses are usually too small for sampling.

The mechanism and the physiological significance of the secretory responses to the various catecholamines are still obscure. Apart from the secretory cells, the mucoid 'dark' cells (Ikai *et al.*, 1970), the myoepithelial cells (Hurley & Witkowski, 1961; Darke & Smaje, 1971) or the ductal cells (Gibson, 1968) may be involved.

Gibson (1968) observed that isoprenaline lowered the Na and Cl content of cholinergic sweat in cystic fibrosis patients. As an explanation of this observation, as well as of the intrinsic sudorific effect of isoprenaline, the author suggested that isoprenaline might inhibit the ductal reabsorption of water. The sweat responses would thus be due to small amounts of unreabsorbed precursor solution reaching the surface. Such an explanation could clarify why the sweat responses to isoprenaline resemble those of spontaneous sweat secretion and why the responses can be blocked by both anticholinergic agents (Gibson, 1968; Warndorff, 1972a) and by propranolol; the former will block the production of precursor fluid, while the latter could prevent the inhibition of its reabsorption.

Ikai *et al.* (1970) observed that α- and β-adrenergic stimuli have opposite effects on the electrolytes of cholinergic sweat. These authors ventured the hypothesis that the sweat glands contain two types of adrenergic apocrine 'dark' cells: α-cells and β-cells, and that the secretion products of these cells influence the reabsorption processes in the ducts.

A stimulatory effect on the 'dark' cells could explain why the sweat responses to adrenergic agents agents are small and unrepeatable; these cells need time for the restoration of their intracellular secretion product.

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Onset of palmar sweating in newborn infants

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SUMMARY

In a study of ninety-one normal newborn infants it has been shown that, contrary to long-standing belief, it is usual to detect eccrine sweating over the palm towards the end of the first day of life. Sweat testing was performed without any form of stimulation, using a 5% solution of *o*-phthaldialdehyde in xylene.

Solomon & Esterley (1970) mentioned that during the first 24–48 h of life, full-term infants usually do not sweat, although sweating begins on the face at about the third day. Referring to Beare & Rook (1968), they commented that palmar sweating begins later. In fact, Beare & Rook (1968), quoting Uchino (1939), stated that palmoplantar sweating may first be provoked by vigorous crying 2–3 months after birth. Although it may be true that loud crying does not commonly increase palmar sweat production until the second month of life or later, we were interested to see whether, in the normal infant, eccrine sweat is produced over the palms on the day of birth or a few days later.

PATIENTS AND METHODS

Eccrine sweat testing over the palms has been performed on ninety-one normal newborn infants (forty-nine males, forty-two females). Eighty-eight of the babies were full-term (i.e. born between 37 and 42 weeks) and the other three were pre-term (all born at 36 weeks). The material used for testing was a 5% solution of *o*-phthaldialdehyde (OPT) in xylene (Juhlin & Shelley, 1967), a safe local application producing no discomfort on application. Neither injections of drugs nor other methods of sweat stimulation were used, and parental consent was obtained before testing. The site selected for testing was the hypothenar area just proximal to the distal transverse crease, and after routinely wiping the palm with gauze a minute amount of solution, which dries quickly, was applied with a brush to a small skin area (about 1 × 2 cm). In the absence of locally applied medicaments which may interfere with the reaction, a black dot with OPT visible at the sweat pore indicates the presence of water and ammonia in the poral infundibulum and represents active sweat production, i.e. a positive reaction; staining does not occur if sweating is completely absent. The result of the test can usually be seen within 2–3 min but the final reading was taken through a magnifying lens 5 or more minutes after OPT application. If only one or two isolated black dots appeared in the test area, the reaction was considered negative. Without stimulation (as in our infants) a negative reaction does not neces-

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sarily indicate non-functioning (or absent) glands, but merely lack of sweating in the particular area at the time of testing.

Prior to testing, the state of the palmar skin was observed. We distinguished between palms that were obviously dry and those that were not, designating the latter as normal, for clarity. However, we of course recognize that slight palmar skin peeling with dryness is not an abnormal finding in newborn babies.

Many of the babies were asleep when tested but none was sedated from maternal anaesthesia. Note was taken of any infants crying at the time of testing. Testing was carried out within the first 6 days after birth but mainly during the first day of life; the youngest babies tested (two) were 2 hours old. Testing was performed in a ward nursery of room temperature 22–30°C (mean 25.3°C). The rectal temperature of five infants at the time of testing was noted (mean 36.9°C).

RESULTS

One hundred and forty tests were performed on the ninety-one infants. The results of the initial test on these infants are shown in Table 1. It can be seen that of seventy-five babies tested on the first day after birth, thirty-two showed a positive reaction and forty-three a negative one. Although no positive reaction was obtained in infants tested within the first 6 h of birth, as the age of the babies tested increased a higher proportion showed a positive reaction. Thus, eighteen (72.0%) of twenty-five babies tested in the final 6 h of their first day demonstrated eccrine sweating. Thirty-seven of the forty-three infants who showed a negative reaction when tested in the first 24 h of life were retested (Table 2), and again the trend to become positive with increasing age is clearly shown.

TABLE 1. Initial *o*-phthaldialdehyde test on ninety-one infants

Day of OPT test	No. of infants	Result	
		Positive	Negative
1st day			
Birth – 6 h	10	Nil (0%)	10 (100%)
7–12 h	22	7 (31·8%)	15 (68·2%)
13–18 h	18	7 (38·9%)	11 (61·1%)
19–24 h	25	18 (72·0%)	7 (28·0%)
2nd day	2	2	Nil
3rd day	4	3	1
4th day	4	3	1
5th day	3	3	Nil
6th day	3	3	Nil

Palmar skin

Although forty-eight of the ninety-one infants showed normal palms when initially tested, the palms of the remaining forty-three were dry. When twenty-eight of the latter infants were retested the palmar skin in six appeared normal. Of the tests performed on dry hands, only thirty (46.2%) out of sixty-five were positive and twenty-three (76.7%) of the positive tests were performed on infants more than 24 hours old. Of the tests performed on normal hands, fifty-nine (78.7%) out of seventy-five were positive, although only thirty-four (57.6%) of these positive tests were performed on infants more than 24 hours old.

Superficial peeling of the palmar skin, associated with dryness, was a common finding and although usually slight was occasionally marked. The OPT findings relating to the state of palmar skin suggest that dry hands are less likely to be producing eccrine sweat. Some of the dryness may in fact be due to lack of eccrine sweating, together with a poorly developed waterholding capacity of the stratum corneum.

Crying

Eight infants were crying at the time of testing (crying was sometimes due to forced unclenching of the clenched fist in order to perform the test). The slight emotional stimulation (if it can be considered as such), which was only transient in all the eight babies, did not appear to affect the results (Table 3).

TABLE 2. Retesting of thirty-seven of forty-three infants negative on initial testing on the first day after birth

Initial test	Retested	No. of infants	Result	
			Positive	Negative
Birth–6 h	2nd day	9	5	4
	3rd day	1	1	Nil
7–12 h	2nd day	9	9	Nil
	3rd day	4	4	Nil
13–18 h	2nd day	5	4	1*
	3rd day	3	2	1
19–24 h	2nd day	6	6	Nil

* This infant was again retested on the sixth day and was positive then.

TABLE 3. OPT test in crying infants

Initial OPT test	No. of infants	Result	
		Positive	Negative
1st day			
7–12 h	2	1	1
13–18 h	2	1	1
19–24 h	1	1	Nil
2nd day	1	1	Nil
3rd day	1	1	Nil
4th day	1	1	Nil

DISCUSSION

Functionally, the eccrine sweat glands can be separated into those of the palms and soles, and those of the general body surface. Those of the palms and soles function continuously, increase their output as a result of mental and emotional stimuli, and respond little, if at all, to heat stress (Cage, 1971). Uchino (1939) studied sweat secretion in the newborn. He found that in seven of eighteen full-term babies, secretion of sweat over palms and soles during loud crying was first seen only between 45 and

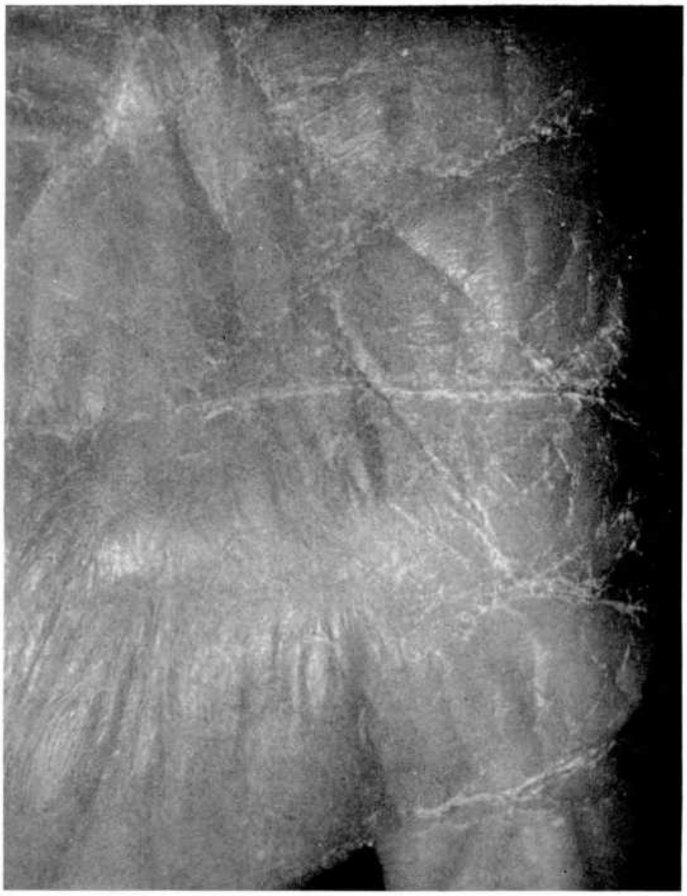

FIGURE 1. Hypothenar area after application of *o*-phthaldialdehyde (5% solution in xylene). Negative reaction (× 7).

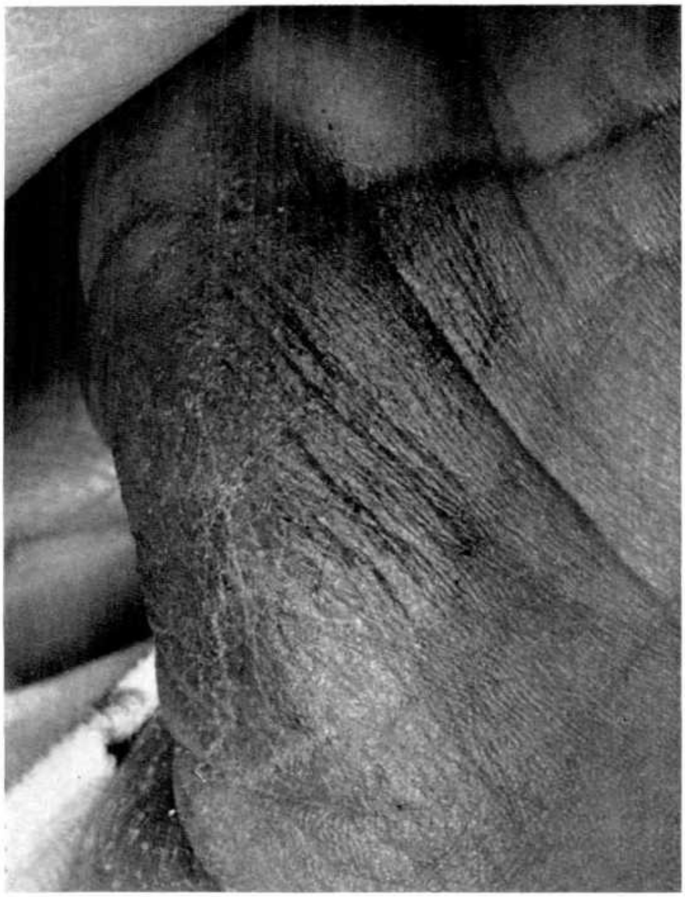

FIGURE 2. Hypothenar area after application of *o*-phthaldialdehyde (5% solution in xylene). Weak positive reaction (× 7).

97 days after birth; in the other eleven infants secretion of sweat was not seen within 25–87 days of birth. He measured the amount of water discharged from a measured area of skin, and when the discharge was distinctly greater on the palms and soles than over a similar area of chest it was concluded that mental sweating was present. Increase in water discharge mostly occurred with crying and struggling, and these increases were regarded as mental sweating. Uchino (1939) used no form of artificial stimulation to provoke sweating. His findings have been accepted down the years as indicating the late onset of palmar and plantar sweating. However, it seems likely that in his study, some of the initial water discharge included eccrine sweat, and vigorous crying served merely to increase the amount of palmoplantar eccrine sweating. We say this in view of our findings in normal (i.e. both non-crying and crying) newborn infants.

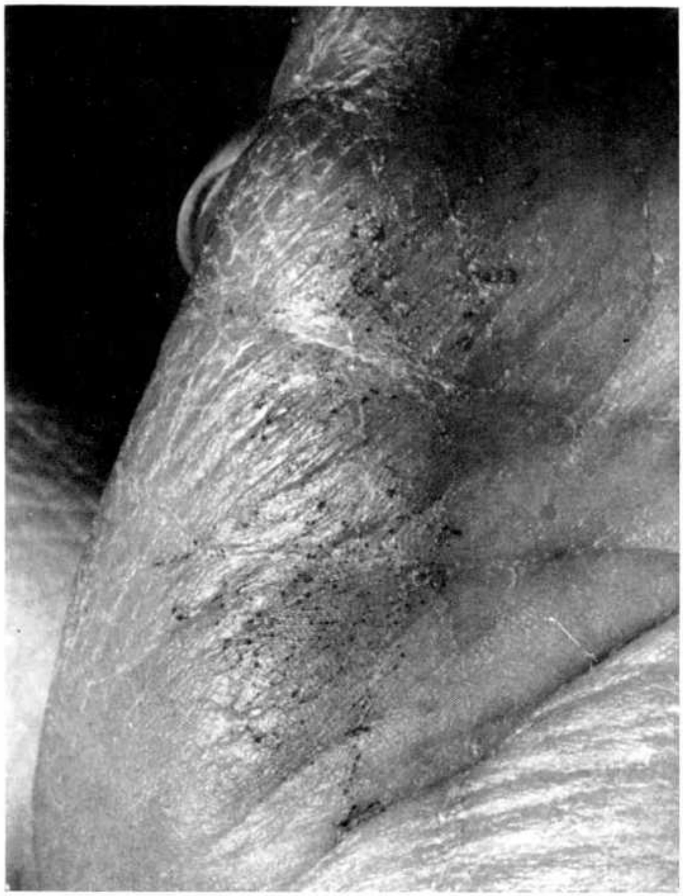

FIGURE 3. Hypothenar area after application of *o*-phthaldialdehyde (5% solution in xylene). Reduced positive reaction (× 7).

To demonstrate eccrine sweating we used a local application, OPT, which requires the presence of water and ammonia in the poral infundibulum to give selective staining of eccrine pores. It can give a positive reaction even in the absence of visible sweat.

Thirty-seven (42.7%) of the seventy-five infants tested on the first day after birth showed evidence of eccrine sweating, and a further thirty-one of those negative at this time demonstrated sweating when retested on the second or third day after birth (Table 2). Thus, by the third day after birth (i.e. more than 48 hours old), sixty-three (84.0%) of the seventy-five infants initially tested on the first day after birth demonstrated eccrine sweating. Of the remaining sixteen infants tested initially between the second and sixth days, fourteen (87.5%) were positive at the time of testing (Table 1).

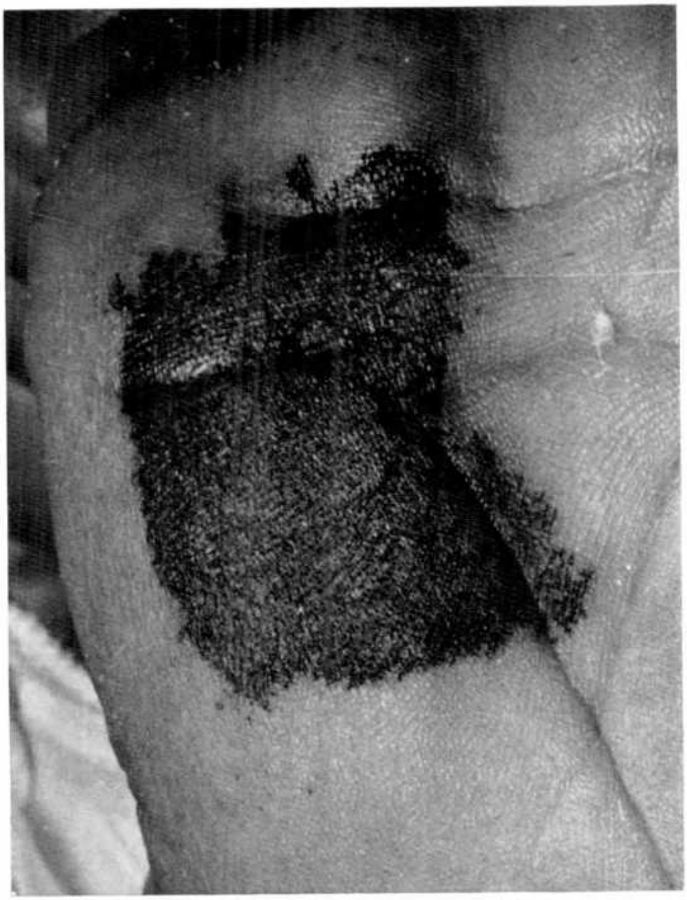

FIGURE 4. Hypothenar area after application of *o*-phthaldialdehyde (5% solution in xylene). Normal positive reaction (× 7).

In the study, a negative reaction was easy to read (Fig. 1), but in positive reactions there was a wide variation in the number of sweat pores selectively stained in the test area. Thus, positive reactions were read as weak, reduced, or normal (Figs. 2–4). In those infants with first day positive reactions retested (on the second or third day), there was a definite trend to increasing positivity of the reaction.

Thus, in normal newborn babies, eccrine sweating can usually be detected over the palm by the third day of life and commonly earlier.

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We should like to thank Professor J.D.Hay for permission to sweat-test the babies.

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Sweat responses of a hyperhidrotic subject

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SUMMARY

Sweating was measured in a subject presenting with palmar hyperhidrosis. The skin of his whole body appeared to sweat excessively in response to mental but not to thermal stress, compared with normal subjects.

A 20-year-old medical student complained of excessive sweating of his hands. This had been present for some years but had become more distressing over the last 3 months, since he had started to examine patients. He did not admit to being a very nervous person, although during conversation about his condition he appeared nervous, flushed and his hands were wet. The following experiments were designed to quantify his sweat responses to various stimuli and to compare them with those of normal subjects.

METHODS

Whole body sweating was estimated by continuously monitoring the rate of total body weight loss in a heat chamber which could be maintained at any desired environmental temperature (Allen, Grimley & Roddie, 1971). Each type of experiment was carried out on the hyperhidrotic subject and on a control group of four or five normal subjects of the same age and sex.

Thermal sweating was measured from the whole body for 60 min periods in environmental temperatures of 18, 29, 34 and 40°C. There was a 60 min equilibration period at the latter two temperatures before steady-state measurements were made. Weight loss was also recorded during the 34°C equilibration period.

Whole body sweat responses to pain and mental stress were studied at 29°C. Pain was induced by occluding the forearm circulation for 5 min following a 1 min period of rhythmic exercise of the forearm muscles. Mental stress was induced by a 10 min period of mental arithmetic and by a 10 min discussion of the subject's academic progress with the Professor of Physiology.

Another procedure was used to study separately the contribution firstly of the skin of the hands and feet and, secondly, of the skin of the rest of the body, to the increase in total body weight loss which occurred during mental arithmetic. All of the body, except the hands, feet, mouth and nose, was covered by plastic bags so that the measured weight loss was derived only from the skin of the hands

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and feet and from the respiratory passages. Then the procedure was reversed, hands and feet being covered by plastic bags and the rest of the body left exposed. Thus the hands and feet no longer contributed to the measured weight loss.

The whole body sweat responses to 15 min intravenous infusions of adrenaline at rates of 5, 10 and 20 μg/min and to 10 min intravenous infusions of pilocarpine at rates of 0·1, 0·2 and 0·4 mg/min were measured at 29°C.

Hand and forearm blood flow responses to mental stress (a 5 min period of mental arithmetic or a 5 min discussion of the subject's recent written examination paper with the Professor of Physiology) and to a 5 min intravenous infusion of adrenaline 5 μg/min were measured by venous occlusion plethysmography.

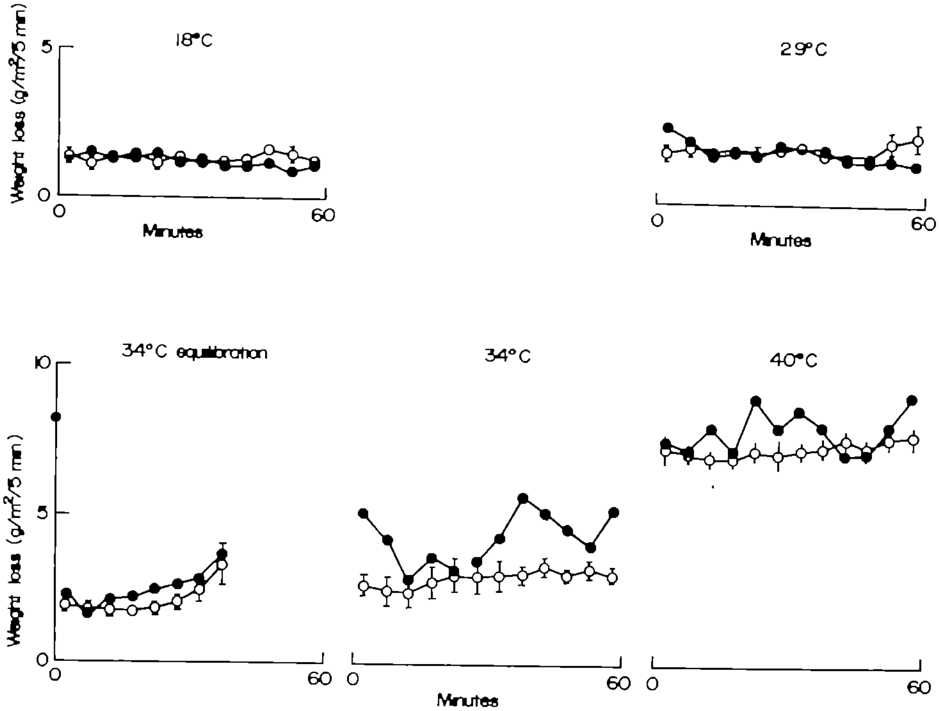

FIGURE 1. Rates of total body weight loss in environmental temperatures of 18, 29, 34 and 40°C. ●, hyperhidrotic subject; ○, mean values (± 1 SEM) of control groups of four or five subjects.

Patterns of heat radiation from the face were studied before, during and after a 10 min period of mental arithmetic using a Thermoscan (EMI Electronics Ltd).

RESULTS

When allowed to relax after reassurance that nothing was going to happen over the period of measurement, the subject did not sweat excessively in environmental temperatures of 18, 29, 34 and 30°C, compared with control observations made on a group of five normal subjects (Fig. 1).

When there were repeated episodes of mental stress or painful stimuli during an experiment, his rate of weight loss was always significantly higher than that of a group of normal subjects, being more than two standard deviations greater than the group mean. During exposures to ischaemic pain, mental arithmetic and discussion of academic progress, his rate of weight loss increased much more than that of a control group of subjects (Fig. 2). The further experiments using the mental arithmetic stimulus indicated that his hands and feet had a high resting rate of evaporation compared with the normal subjects, although the rest of his body did not. The results also showed that the skin of both his hands and feet and of the rest of his body responded excessively to the stressful stimulus compared with a control group of normal subjects.

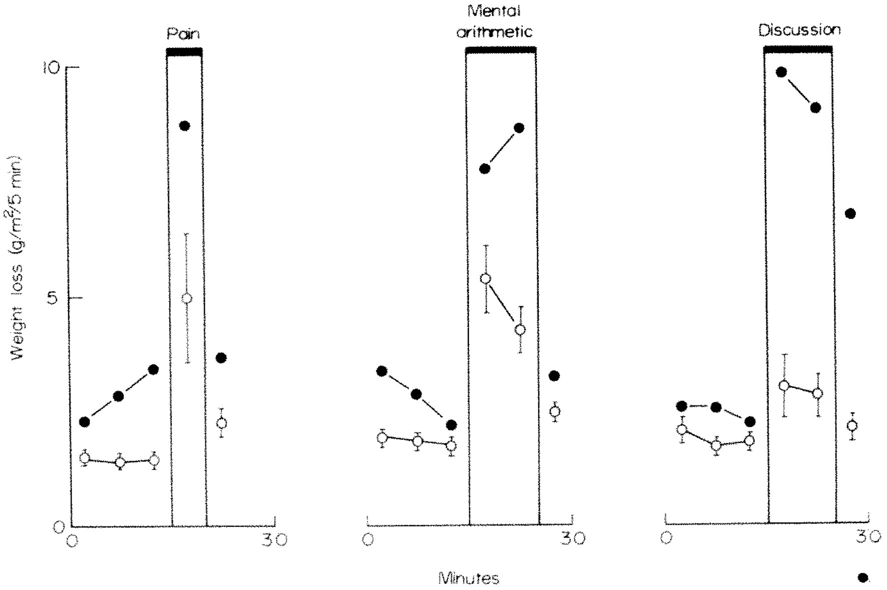

FIGURE 2. The effects of ischaemic pain, mental arithmetic and discussion of academic progress on the rate of total body weight loss. ●, hyperhidrotic subject; ○, mean responses (± 1 SEM) of a group of control subjects.

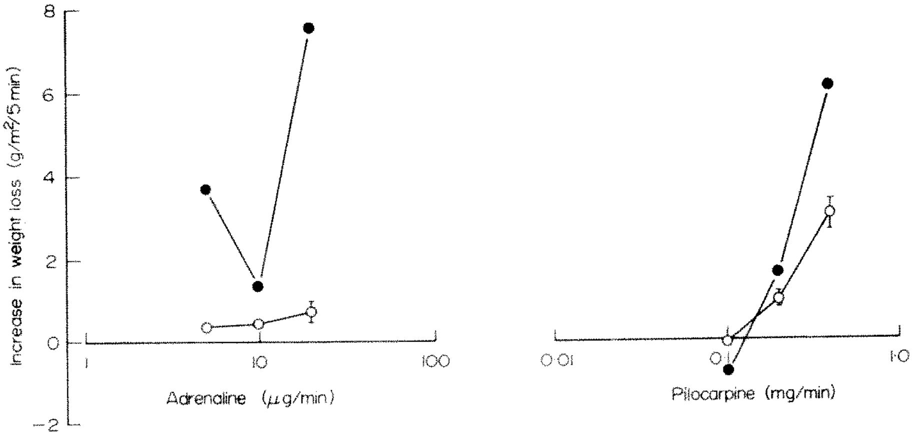

FIGURE 3. Dose response curves for the sweat responses to intravenous infusions of adrenaline and pilocarpine. ●, hyperhidrotic subject; ○, mean responses (± 1 SEM) of a control group of five subjects.

The dose response curves constructed for his sweat responses to adrenaline and pilocarpine indicated that during infusion of these drugs the subject sweated more than normal people (Fig. 3).

Resting forearm and hand blood flows in the hyperhidrotic subject were 3·7 (SE ±0·1) and 5·1 (±0·8) ml/100 ml/min respectively. During adrenaline infusion at a rate of 5 μg/min, they were 6·1 (±0·6) and 4·8 (±0·8) ml/100 ml/min. During mental arithmetic and discussion of examination papers, forearm flow rose from control values of 3·6 (±0·1) and 3·2 (±0·1) to mean values of 6·8 (±0·3) and 6·0 (±0·4) ml/100 ml/min respectively, while the corresponding hand flows decreased from control values of 3·8 (±0·4) and 7·5 (±0·9) to 2·8 (±0·3) and 2·8 (±0·2) ml/100 ml/min. None of these responses could be considered to be excessive.

During mental arithmetic, heat emission from the face decreased generally, the response being most marked in the nose and lips. This decrease was apparent in the presence of marked facial flushing and absence of visible sweating. Similar changes were observed in four control subjects.

DISCUSSION

Hyperhidrosis is excessive production of sweat. If thermoregulatory sweating is excessive, it may occur especially on the trunk and face or over the general body surface (Champion, 1970). However, essential hyperhidrosis is thought usually to have an emotional basis and is most commonly restricted to the palms and soles and possibly the axillae, although sometimes it occurs over the general body surface.

The present experiments suggested that, in this subject, thermal stimuli did not cause excessive sweating and that his hyperhidrosis was related to anxiety or mental stress. It appeared to be generalized in that although only his hands and feet had a high resting rate of evaporation, in stressful situations the rest of his body also over-responded compared with control subjects. The palms and soles have been thought to be the main sites of mental sweating in normal people (Kuno, 1956), but under our experimental conditions the skin of all regions appears to participate in mentally induced sweating (Armstrong, 1973).

The sweat responses to both adrenaline and pilocarpine infusions were very large. However, at doses producing a sweat response, the subject is subjectively aware of the cardiac effects of the adrenaline infusion, and the hyperhidrotic subject's sweat responses may therefore be due to an increased emotional response rather than to increased sensitivity to adrenaline. The shape of the adrenaline dose response curve would support this suggestion. The initial dose of 5 μg/min induced a greater response than the next dose of 10 μg/min, possibly because having once encountered the drug, the subject was not so distressed by it the next time. Pilocarpine produces sweating by a ganglionic as well as a peripheral action. The subject is again made subjectively aware of the infusion of the two largest doses used in these experiments by tachycardia and salivation, and so the hyperhidrotic subject's sweat responses to pilocarpine probably do not indicate increased sensitivity of the sweat glands. His large responses are probably not related to the process of intravenous infusion itself, as this was continuous throughout these experiments, saline being infused between the different doses of the drugs.

Increased reactivity of the sympathetic nervous system has been described as a feature of hyperhidrosis, but in this subject the peripheral vascular responses to adrenaline infusion and mental stress appeared to be within normal limits. There was no evidence of the vasodilatation in the hand in response to mental arithmetic observed by Allwood *et al.* (1959).

Decreased heat emission from the face in the presence of flushing, and therefore presumably vasodilation, suggests that cooling due to evaporation of sweat was sufficient to more than counteract the vascular changes. However, a similar pattern was found in normal control subjects.

In conclusion, the subject's hyperhidrosis appeared to be a generalized condition, although he was aware of it only on his hands. It seemed to be a manifestation of his over-reaction to stressful situations.

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Changes in the T-wave of the electrocardiograph in urticaria

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SUMMARY

A series of cases is presented, in which non-specific primary changes in the T-waves were observed in electrocardiographs of patients with urticaria, the latter caused by drugs or other substances. In all these patients, no other abnormality was found and they were completely asymptomatic as regards cardiac involvement.

A definite 'cause and effect' type of relationship was found between the T-wave changes and the urticaria. The mechanism of the T-wave changes remains to be investigated.

Since this phenomenon disappears simultaneously with the urticaria, treatment should be determined by the urticaria, and bed rest is not indicated.

Numerous factors are known to affect the T-waves in the electrocardiograph. These may be classified as primary or secondary.

In this report we present a series of cases in which non-specific primary changes in the T-waves were observed in patients with urticaria caused by drugs or other substances. These changes were strictly confined to the T-waves and disappeared completely with the disappearance of the urticaria. In none of these cases were there any cardiac symptoms, and physical examination and laboratory tests, including various enzyme assays, showed no deviation from the normal.

It is well known that various drugs may affect the heart, and particularly the T-waves (Friedberg, 1969, a). A specific nosological entity called 'allergic myocarditis' has been described (French & Weller, 1942); in these patients ECG changes compatible with myocarditis were observed, but in all of them the final diagnosis was determined at post-mortem. In contrast, our cases manifested a hypersensitivity reaction which caused no permanent damage to the heart, the ECG changes disappearing within 2–8 days from the time of their appearance.

To the best of our knowledge there are few references to this phenomenon in the medical literature (Criep, 1931; Bullrich, 1939; Foster & Laylan, 1952; Rogen & Richardson, 1961).

CASE REPORTS

For the purpose of this study, the files of all patients admitted to our hospital in the last 10 years with the diagnosis of urticaria were reviewed. Among 102 such cases, and in whom an ECG was done routinely, there were seven in which all the other aetiological factors, which might possibly explain the ECG changes, could be excluded.

Case 1

A 13-year-old girl was first hospitalized on 23 June 1968 because of an urticarial rash accompanied by a fever of 39°C. She was known to suffer from hypersensitivity to various substances.

No abnormality was found, either on clinical examination or in the laboratory tests. The chest X-ray was normal. On the day of admission the ECG showed flattening of the T-waves in leads I, II, AVR, V_{5-6} and inversion of the T-waves in leads III, AVF and V_{1-4}. These changes had disappeared by the time of her discharge 5 days later (Fig. 1).

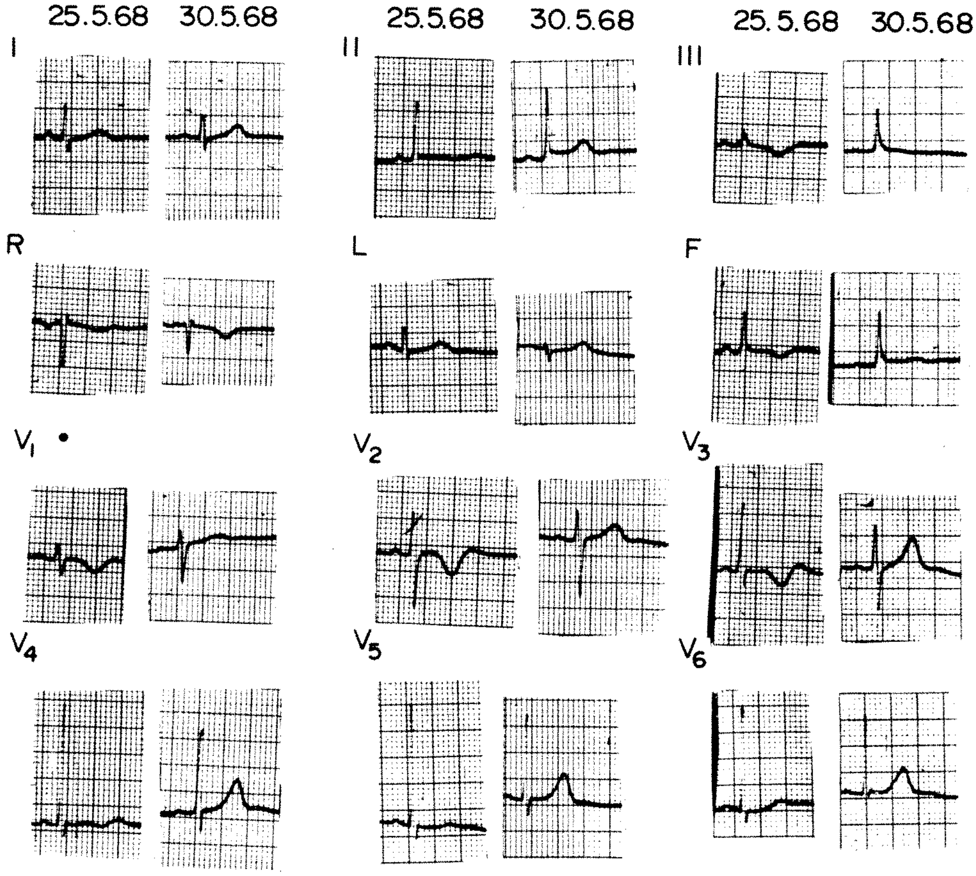

FIGURE 1. T-wave changes in urticaria in a 13-year-old girl, which disappeared 8 days later.

She was again hospitalized on 6 May 1969 with the same clinical picture, and with no pathological findings either on physical examination or in the laboratory tests. The changes in the ECG were identical to those recorded during the previous hospitalization and disappeared within 5 days.

The third and last hospitalization on 5 June 1972 was similar, except that the ECG changes disappeared after 6 days.

Case 2

A 21-year-old woman was admitted with an urticarial rash brought on by penbritin given for an upper respiratory infection. The physical examination and laboratory tests showed no pathological findings. On admission, the following changes were observed in the ECG; flattening of the T-waves in leads I, II, III, AVR, AVL, AVF and V_{4-6}, and inversion of the T-waves in V_{1-3} (Fig. 2). These changes disappeared 8 days later, together with the urticaria and accompanying pruritus.

Case 3

A 14-year-old girl was admitted because of urticaria and a fever of 38°C. The ECG showed flattened T-waves in leads I, II, AVR, AVL and V_3, and inversion in III, AVF (which failed to disappear in the recording made during deep inspiration) and V_{1-5} (Fig. 3).

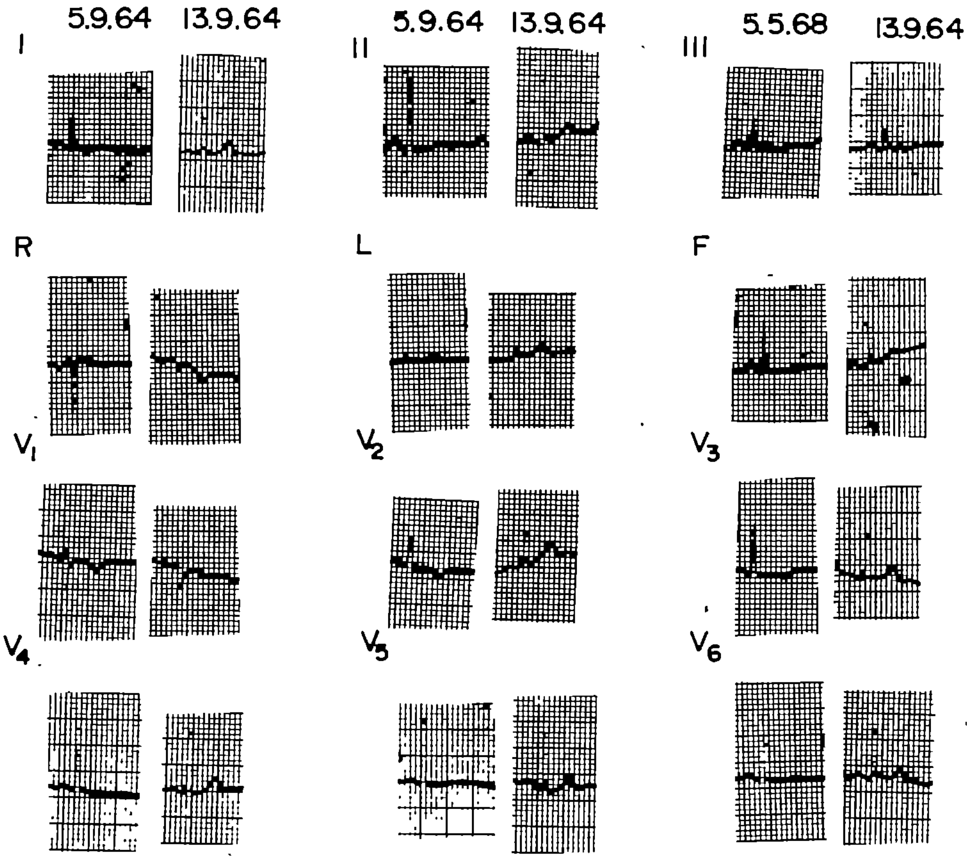

FIGURE 2. T-wave changes in urticaria in a 21-year-old woman, which disappeared 2 days later.

Case 4

A 22-year-old woman was admitted with urticaria which had developed after she had been stung on the knee by an insect. Physical and laboratory examinations showed no deviation from the normal. The ECG on her admission showed flattened T-waves in AVF and inverted T-waves in III and V_{1-4}, which returned to normal 7 days later.

Case 5

A 38-year-old woman was admitted because of urticaria which developed after the administration of salicylates. The ECG recorded the day after her admission showed flattening of the T-waves in all other leads, but inversion in III, AVF and V_{1-4}, with a return to normal 2 days later.

Case 6

This 47-year-old woman was known to be suffering from mitral stenosis and had undergone a commissurotomy 6 years before. She was being given quinidine, deralin, hydantoin and phenobarbitone.

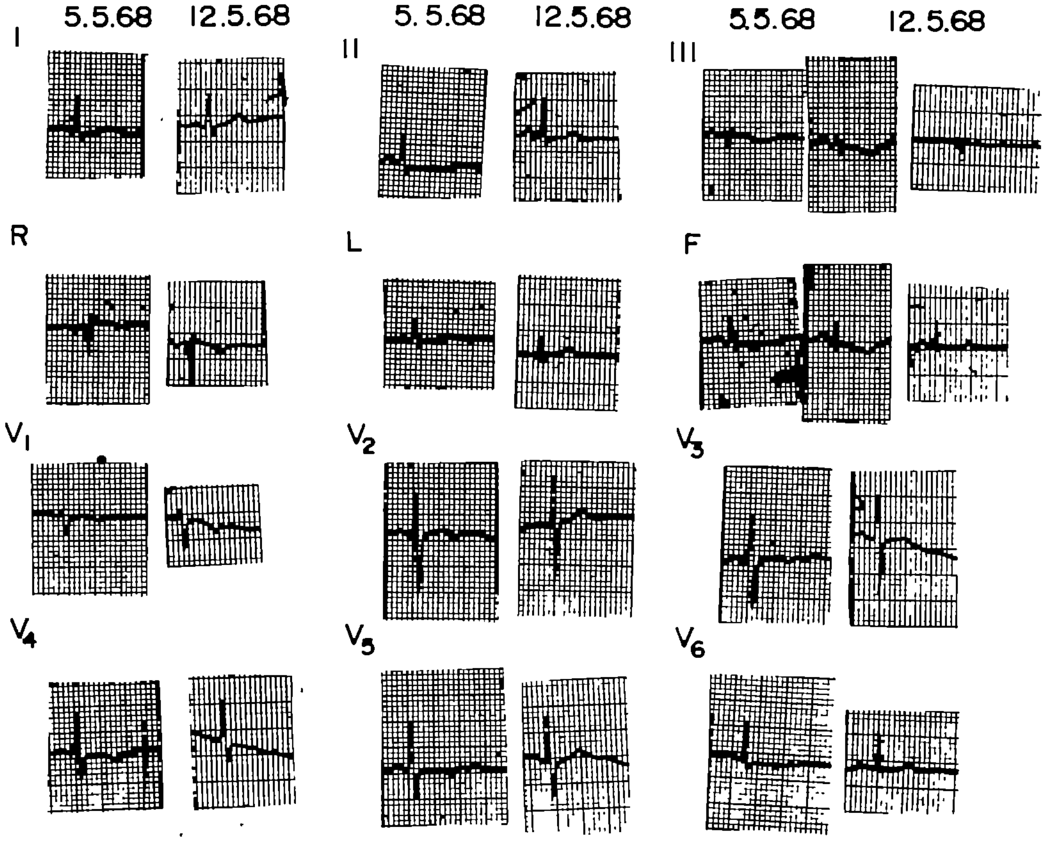

FIGURE 3. T-wave changes in urticaria in a 14-year-old girl, which disappeared 7 days later.

She was admitted with an urticarial rash accompanied by changes in the ECG which consisted of inversion of the T-waves in II, III, AVF and V_{1-3}, and flattening in I, AVR and V_{5-6}, with a return to normal 2 days later. Whilst she was in hospital these drugs were not stopped; the hypersensitivity reaction was found to have been caused by penbritin given for an upper respiratory infection.

Case 7

This 53-year-old male was admitted because of an urticarial rash which had developed after the administration of erythromycin. The ECG showed flattening of the T-waves in all other chest leads, but an inversion in V_{1-3}. After an infusion of ACTH he suffered a transient episode of sinus tachycardia which was treated with digoxin and inderal. On his discharge 3 days after admission, the T-waves had become positive in all leads. The physical and laboratory examinations showed no deviation from the normal.

All these patients were given an infusion of 40 units of ACTH on admission and pyribenzamine tablets, 100 mg × 3 daily.

For the purpose of this study, a histamine test was carried out in nine healthy controls and in one of the patients in this series (case 1). 0·5 mg of histamine was injected subcutaneously and ECG recordings were made 10, 30 and 60 min later. No changes were found in any of the test subjects.

TABLE 1. The cases which presented ECG changes

Case no.	Sex	Age (years)	Cause of urticaria	ECG changes	Time elapsed until return to normal (days)
1	F	14	General hypersensitivity. Did not receive any medications	Flat T—I, II, AVR, V_{5-6} Inverted T—V_{1-4}	5–6
2	F	21	Penbritin (upper respiratory infection)	Flat T—AVR, AVL, AVF, V_{4-6}, I, II, III Inverted T—V_{1-3}	
3	F	14	Cause unknown. Did not receive any medications	Flat T—AVR, AVF, V_6, I, II Inverted T—III, AVF, V_{1-5}	7
4	F	22	Insect sting	Flat T—AVF Inverted T—III, V_{1-4}	7
5	F	38	Salicylates (headache)	Flat T—AVR, AVL, V_{5-6}, I, II Inverted T—II, AVF, V_{1-4}	2
6	F	47	Penbritin (upper respiratory infection)	Flat T—I, AVR, V_{5-6} Inverted T—AVF, V_{1-3}, II, III	2
7	M	53	Erythromycin (abscess on the neck)	Flat T—AVL, AVF, V_{4-5}, I, II, III Inverted T—V_{1-3}	3

DISCUSSION

Primary or secondary T-wave changes seen in the ECG may be specific or non-specific. Secondary T-wave changes are secondary to changes in the height and duration of the QRS complex, which in turn are due to alterations in the course of depolarization. If, however, there are fundamental metabolic changes in the muscle, as a result of changes in blood supply or other injuries, there are alterations in the differences of duration and intensity of depolarization and the course of repolarization, i.e. the ventricular gradient, is altered. A negative T-wave due to such an altered course of repolarization and not to an alteration in depolarization (QRS), is termed a primary T-wave change (Friedberg, 1969, b).

Specific changes are those in which the reason or cause for the change can be defined, e.g. ischaemia, haemodynamic factors or electrolyte changes. In contrast, non-specific changes are those in which the cause for the change in T-waves cannot be determined. In this case, other clinical factors must be taken into consideration in addition to the ECG (Friedberg, 1969, c).

In this paper we have presented a series of cases suffering from urticaria, all of whom showed non-specific primary T-wave changes.

A number of factors are known to affect the T-waves in the ECG. These include smoking, drinking cold water (Sears & Manning, 1958), hyperventilation (Wasserburger *et al.*, 1956), cyclic inversion of the T-waves during menstruation (Edeiben, 1962), the ingestion of a large meal (Sears & Manning, 1958) and body posture (Friedberg, 1969, b).

We would now like to add another factor to this list.

The age range of the patients in this series was from 14 to 53 years. All of them had been admitted with the diagnosis of urticaria, and in all of them, with one exception (case 6), there was no clinical, laboratory or radiological finding which might have indicated cardiac disease of any type. A finding common to all these cases was the appearance of T-wave changes in the routinely performed ECG, either on the day of admission or the day after. There was flattening or inversion of T-waves in various leads (Table 1) in which these changes are not ordinarily seen. In doubtful cases, we excluded all of the above-listed aetiological factors (e.g. smoking, etc.) which are known to cause this type of change. We furthermore excluded from this series all those cases in which there was still any doubt as to the aetiology.

The T-wave changes disappeared completely after 2–8 days. This interval paralleled the intensity of the urticaria.

It must be stressed, that throughout the hospitalization, all these patients were completely asymptomatic as regards cardiac involvement. The male–female ratio was 1:6, which was similar to the ratio found in the total number of cases with urticaria (twenty-two males, eighty females).

In our opinion, a definite 'cause and effect' type of relationship can be seen between the T-waves and the urticaria, since the appearance of the T-wave changes was contingent upon the development of the urticaria and since their disappearance paralleled the disappearance of the urticaria. The most striking support of this assumption is presented by case 1. This young girl suffered three separate episodes of urticaria at more or less yearly intervals, each time presenting identical changes in the ECG, which disappeared at the end of the attack.

In an attempt to find the mechanism of the T-wave changes, we assumed that they might originate from the effect of histamine, which is well-known to be one of the principal substances liberated from mast cells during the urticarial process (Harrison, 1970). We therefore performed a histamine test, consisting of the subcutaneous injection of 0·5 mg histamine, in nine healthy subjects and in one of the cases in the present series (case 1). ECG recordings made previous to the injection and 10, 30 and 60 min afterwards showed no deviation from the normal. The mechanism for these changes thus remains to be investigated.

In the light of our experience with these cases, we should like to stress that these T-wave changes should not be treated as though they indicate chronic ischaemia or acute myocarditis. There is no need to confine these patients to bed, since this phenomenon disappears simultaneously with the urticaria. Treatment should be determined by their basic disease, i.e. the urticaria.

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Heat urticaria

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SUMMARY

A case is reported of urticaria as a local response to heating the skin. The clinical, pharmacological and immunological features of this rare condition are described.

Urticaria as a local response to heating of skin is extremely rare. We describe here a patient with localized heat urticaria, and report some clinical, pharmacological and immunological characteristics of the condition.

CASE REPORT

The patient is a 36-year-old married woman, who first attended the Rupert Hallam Department of Dermatology, Sheffield, in September 1971, complaining of an itchy rash in heat-exposed skin, which had developed a few weeks after the birth of her first and only child 3 years previously. She was otherwise healthy. The rash was provoked by hot baths, radiant heat, and contact with hot cooking utensils, and was precisely confined to the skin exposed to heat. No other form of physical stimulus caused an abnormal response in the skin, and exertion and emotion did not cause relapses. Physical examination was negative and there was no dermographism on firm stroking of the skin.

Wealing evoked by heat

Skin was heated by placing a beaker of warm water on the forearm. Redness and oedema immediately developed, which were confined to the area of contact with the base of the beaker. There was no axon reflex flare. The threshold temperature for urtication was 43°C and wealing was maximal at 49°C. At this temperature, skin had to be warmed for at least 1 min to produce wealing, and wealing was maximal after warming for 5 min. The weal began to fade after 15 min. The wealing was not a local form of cholinergic urticaria because intradermal injection of atropine did not inhibit it. Responses of uninvolved skin to histamine, bradykinin, serotonin, acetylcholine, prostaglandin E_1 and compound 48/80 were within normal limits.

Histamine

Systemic administration of an anti-histamine (chlorpheniramine) caused diminution of wealing but did not affect erythema. A similar inhibitory effect was produced locally by intradermal injection of chlorpheniramine. Further evidence that histamine is involved in heat urticaria was obtained by using an

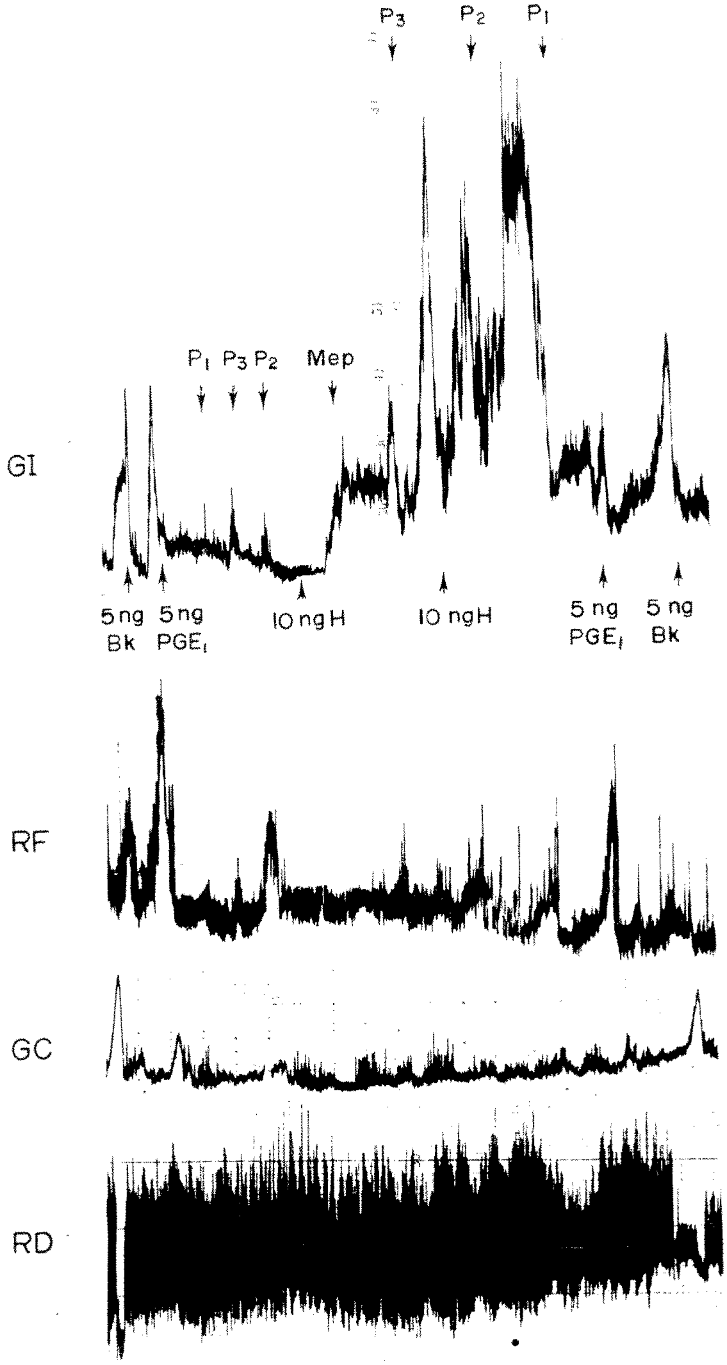

FIGURE 1. Responses of four isolated smooth muscle preparations to perfusates from urticated skin. GI, guinea pig ileum; RF, rat stomach fundus; GC, gerbil colon; RD, rat duodenum. P_1, P_2, P_3, perfusates obtained 5, 10 and 15 min after heating perfused skin. GI gives contractile responses to P_1, P_2 and P_3. Both these contractions and the contractile response to 10 ng histamine (10 ng H) are abolished by the histamine antagonist mepyramine 0·1 μg/ml (Mep). Bradykinin (Bk) and prostaglandin E_1 (PGE_1) produce normal contractile responses of the GI both before and after Mep. RF gives small contractile responses to P_1, P_2 and P_3 which are not abolished by mepyramine. GC gives contractile response to bradykinin and prostaglandin E_1 but not to perfusates. RD relaxes in response to bradykinin but is unaffected by the perfusates.

arterial occlusion method on the forearm (Greaves & Shuster, 1967) in which cross-tachyphylaxis between heat urticaria and histamine wealing was demonstrated.

Skin which had previously urticated in response to heat remained refractory to heat for at least 3 weeks, although there was no corresponding prolonged refractoriness of the same skin to histamine.

The presence of histamine in the heat-urticated skin was confirmed by skin perfusion (Greaves, Sondergaard & McDonald-Gibson, 1971) in which perfusates, obtained *in vivo* from heat urticated skin, were cascaded over a series of different isolated smooth muscle preparation continuously moistened by Tyrode solution at 37°C. Perfusates obtained from skin after but not before heating the perfused skin contained histamine (Fig. 1).

Other pharmacological activity

Perfusates from heat-urticated skin, but not uninvolved skin, caused a contraction of the rat stomach fundus preparation (Fig. 1), which was not antagonized by atropine, mepyramine or bromlysergic acid diethylamide, and thus contained no acetylcholine, histamine or serotonin. The effect was not due to kinin activity since it did not cause relaxation of the rat duodenum. Its identity is uncertain.

Effect of heat on basophil leukocytes

Warming the patient's leukocytes *in vitro* to 49°C did not evoke significant histamine release. Leukocytes were separated from heparinized blood using 1% methylcellulose to accelerate sedimentation of the red cells. Triplicate aliquots of washed basophil-rich leukocyte suspensions were warmed for 15 min at temperatures ranging from 37 to 49°C. A parallel control experiment was carried out using washed leukocyte suspensions from an apparently healthy subject. There was no significant difference between histamine releases from basophils of the two donors.

Immunological studies

Serum immunoelectrophoresis and determination of serum immunoglobulins by radial immunodiffusion did not reveal any obvious quantitative abnormality of IgA and IgG, although the concentration of IgM was increased (229 mg/100 ml, normal range 47–170). The total serum IgE concentration was 127 International Units per ml (normal range, 25–375 U/ml). There was no evidence of circulating immune complexes in the serum (Dr J. J. Cream, Middlesex Hospital, London). Warming the patient's serum to 49°C did not result in visible evidence of pyroglobulins. Attempts were made to demonstrate passive transfer of heat urticaria using the patient's serum. Skin of baboons was injected intradermally in triplicate with both undiluted serum and a 1:5 dilution of serum. After 4 and 24 h the injected sites were challenged by heating at 48°C for 1 min. Positive and negative control experiments were carried out simultaneously in the same animals by injecting a serum (dilution 1:10) from a pollen-sensitive individual. Sites injected with the reaginic serum gave a positive reaction on challenge with Timothy pollen antigen. Heat challenge of both test and saline injection sites gave negative results at 4 and 24 h. Thus no evidence of a transferable serum factor was obtained in these experiments.

DISCUSSION

We have found reports of four patients with heat urticaria in the literature over the last 50 years (Hopkins, Kesten & Hazel, 1938; Baer & Harber, 1965; Delorme, 1969). Michaelson & Ros (1971) recently described a family with delayed cutaneous inflammation in response to heat which might be related to heat urticaria. None of these case reports shed light on the mechanism of heat urticaria. By use of specific histamine antagonists and by demonstrating cross-tachyphylaxis with histamine we have produced indirect evidence that heat urticaria is mediated by histamine. We also have direct evidence, since histamine was directly recovered by perfusion of urticated skin. The development of a refractory state of heat urticaria in previously heat-urticated skin lasting for 3 weeks could be explained by the time taken for replenishment of histamine-depleted stores by biosynthesis in skin mast cells. A refractory state of similar duration develops in the Prausnitz–Küstner reaction (Bowman & Walzer, 1953). That histamine is not the only mediator of the vascular changes of heat urticaria is suggested by the presence of non-histamine smooth muscle-contracting activity in perfused urticated skin which was not due to acetylcholine, serotonin or bradykinin. This activity might account for the erythematous component of heat urticaria which was not inhibited by chlorpheniramine.

Histamine release is characteristic of immediate hypersensitivity reactions in skin (Sondergaard & Greaves, 1971), but an allergic basis for heat urticaria seems unlikely in the present patient. There was no personal or family history of atopy and prick tests to a wide range of common antigens were negative. The total serum IgE concentration was also normal. Solar urticaria and cold urticaria can sometimes be passively transferred by serum to the skin of a healthy recipient (Sams, Epstein & Winkelmann, 1969; Houser *et al.*, 1970), but the present patient's serum did not confer abnormal heat-reactivity on recipient baboon skin. Delorme (1969) came to a similar conclusion. The skin blood vessels in the patient responded normally to vasoactive agents. Thus, a non-allergic mechanism, leading to histamine release, seems probable in heat urticaria. The failure of heat to evoke abnormal histamine release from the patient's basophil leukocytes *in vitro* suggests the interaction of heat with a tissue or plasma factor, leading to activation of an enzymic histamine release cascade or perhaps more simply to disruption of the skin mast cell membrane.

ACKNOWLEDGMENTS

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Granuloma pyogenicum—a hitherto unrecognized complication of smallpox vaccination

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SUMMARY

Nineteen cases of granuloma pyogenicum, arising at the site of smallpox vaccination, are reported The cause of this lesion, which remains obscure, is discussed and the possibility that an infective agent is an aetiological factor is suggested.

A variety of complications of smallpox vaccination are known to occur. Neff *et al.* (1967) and Lane *et al.* (1969) carried out extensive national surveys on the occurrence of such complications after mass vaccination in the United States. These included postvaccinial encephalitis, vaccinia necrosum, eczema vaccinatum, generalized vaccinia and a few rarer complications. The occurrence of granuloma pyogenicum was not noted by these workers and has not, so far, been reported.

In a recent mass campaign of smallpox vaccination in Jordan, we encountered nineteen cases of granuloma pyogenicum arising at the site of vaccination. In this paper we report this new observation and discuss the aetiology of this lesion.

MATERIALS AND METHODS

In April 1972, smallpox was reported in neighbouring Iraq and Syria. Consequently, the Ministry of Health and the Royal Army Medical Service (RAMS) in Jordan carried out mass vaccination of about 70% of the country's population. Approximately one million people were vaccinated, including 300,000 civilian and military personnel, who were vaccinated at this hospital and other RAMS associated centres.

The vaccine was prepared at the Laboratories of the Jordan Institute of Vaccine Preparation (JIVP) under the sponsorship of the World Health Organization (WHO). The skin of sheep is used for harvesting the vaccine, the seed of which is maintained by alternate propagation in the skin of sheep and rabbits. The vaccine is processed, and prepared in liquid form, in accordance with standards recommended by the WHO (1966).

The vaccination was carried out at many hospitals and clinics all over the country, on the outer aspect of the upper arm, using the multiple scratch technique. The same batch of vaccine was used throughout this campaign.

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During the following weeks we saw, at this hospital, nineteen typical examples of granuloma pyogenicum arising at the site of vaccination. One patient (case 19) was treated conservatively. In the remaining patients (cases 1–18), the lesions were excised. The tissue was fixed in 15% formol-saline, and embedded in paraffin. Sections were stained with haematoxylin and eosin and with a variety of special stains. These included Periodic acid-Schiff reaction (PAS) with and without previous diastase digestion, Gram's stain, Masson's trichrome for collagen, Wilder's preparation for reticulin, Weigert's stain for elastic and Perls' Prussian blue reaction for haemosiderin.

Later, tissue was obtained from formalin-fixed and paraffin-embedded blocks, from two cases (cases 3 and 5), postfixed in osmic acid, dehydrated and embedded in epoxy resin (Araldite). Thin sections were stained with uranyl acetate and lead citrate, and examined in a Philips EM 300 electron microscope.

Dr R. Kamal (1972), Director of the JIVP, carried out a bacteriological culture on aspirated material from one lesion and on a sample from the same batch of the vaccine and obtained no growth. Virological cultures could not be prepared.

CLINICAL FEATURES

The pertinent clinical details of the nineteen cases are seen in Table 1. No other relevant findings were obtained from their clinical history or physical examination. In none of the patients was there any evidence of hypogammaglobulinaemia or of any other immunological deficiency disorder. The lesion in case 19, which was treated conservatively, disappeared spontaneously 3 weeks after onset. No other unusual sequelae were observed in the rest of the vaccinated population.

TABLE 1. Clinical data of cases

Case no.	Initials	Sex	Age (years)	Duration between vaccination and eruption of lesion (days)	Diameter of lesion (mm)
1	M.S.	M	24	7	5
2	A.H.	M	26	10	6
3	S.H.	F	25	7	15
4	H.M.	M	30	12	10
5	R.S.	M	8	8	5
6	S.F.	F	7	14	10
7	S.R.	F	21	8	8
8	K.H.	M	47	20	5
9	S.S.	M	9	7	5
10	M.A.	M	22	10	10
11	I.B.	F	23	15	18
12	A.M.	M	45	20	10
13	W.H.	M	28	10	5
14	H.A.	F	46	20	15
15	M.K.	M	20	20	5
16	A.H.	F	14	8	15
17	A.E.	M	7	7	5
18	I.E.	M	4	7	10
19	N.Q.	M	9	21	10

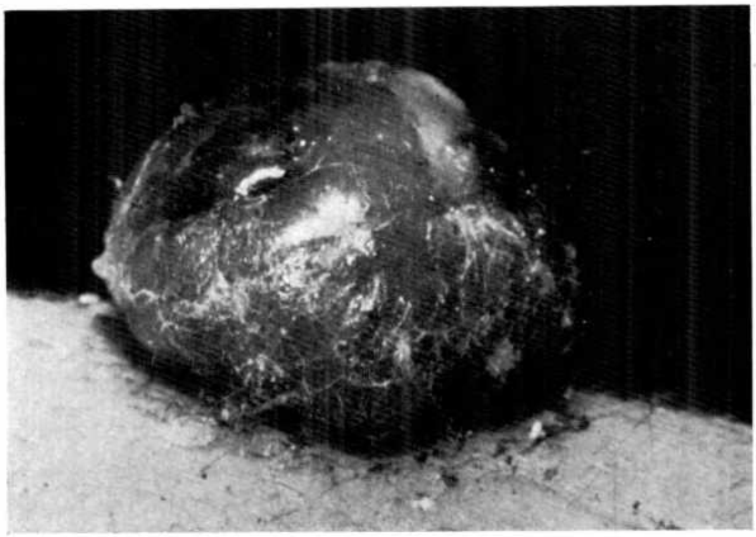

FIGURE 1. Photograph of lesion on arm (case 3), showing round, lobulated and somewhat pedunculated nodule.

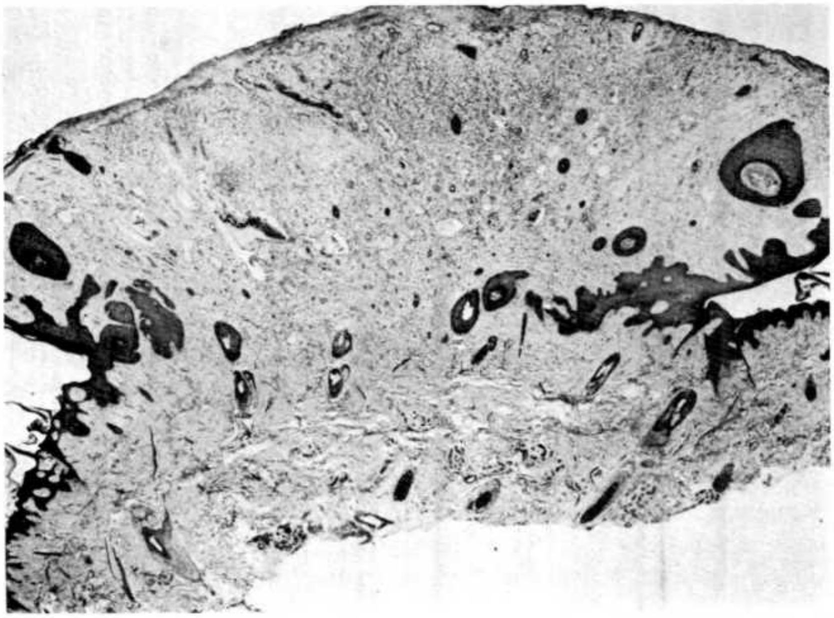

FIGURE 2. Case 5, low power photomicrograph showing the epidermal collarette and hyperplasia at the base of the lesion (H & E, × 14).

The lesions were in all cases clinically very similar. The nodules were painless and bled easily. They were bright red to brownish-red in colour, raspberry-like, lobulated and globular with a somewhat-pedunculated base (Fig. 1); a few lesions were sessile.

PATHOLOGICAL FINDINGS

Histologically, the lesions were very similar and hence may be described together. They were elevated and well circumscribed. In many lesions, the epidermis was centrally thinned and ulcerated. The ulcerated portion was covered with a fibrinous exudate. At the border, there was epidermal hyperplasia with inward growth, forming at the base the typical epidermal collarette (Fig. 2). In the dermis, mainly superficially, there was a moderate inflammatory infiltrate composed of lymphocytes, plasma cells, polymorphs and few eosinophils. The nodules contained numerous newly-formed capillaries of varying size, lined by an increased number of prominent endothelial cells, set in a loose oedematous stroma with active fibroblastic proliferation in some areas (Figs. 3 and 4).

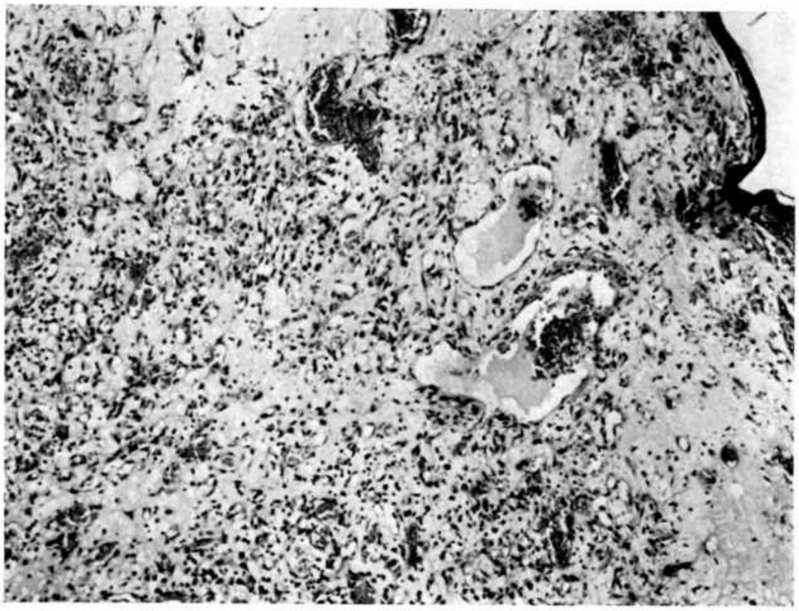

FIGURE 3. Case 3, showing oedematous stroma and preponderance of angiomatous element with ectatic vessels (H & E, × 80).

No haemosiderin pigment was noted and the elastic tissue in the nodules was markedly diminished or absent. Perivascular reticulin networks were seen, but little or no mature collagen was present. Gram stain revealed small clusters of Gram-positive cocci in the fibrinous exudate, covering the surface of some of the ulcerated lesions, but no fungi were recognized. No intracytoplasmic inclusions were seen.

Electron microscopy revealed no viral particles or inclusions, but it must be taken into consideration that these sections were of poor quality as a result of the previous formalin-fixation and paraffin-embedding.

COMMENT ON THE HISTOLOGY

The histological appearances of these lesions were those usually seen in granuloma pyogenicum. However, in view of the unusual clinical background and the possibility of a disease transmitted from animals, through the administered vaccine, sections from some of these lesions were reviewed by several noted pathologists, who confirmed the diagnosis of granuloma pyogenicum (Azzopardi, 1972; Whimster, 1972). Cotchin (1972) did not think these lesions were the same as those he described in cattle as 'bovine cutaneous angiomatosis' (Cotchin & Swarbrick, 1963), nor did he consider contagious pustular dermatitis (orf) as a likely diagnosis.

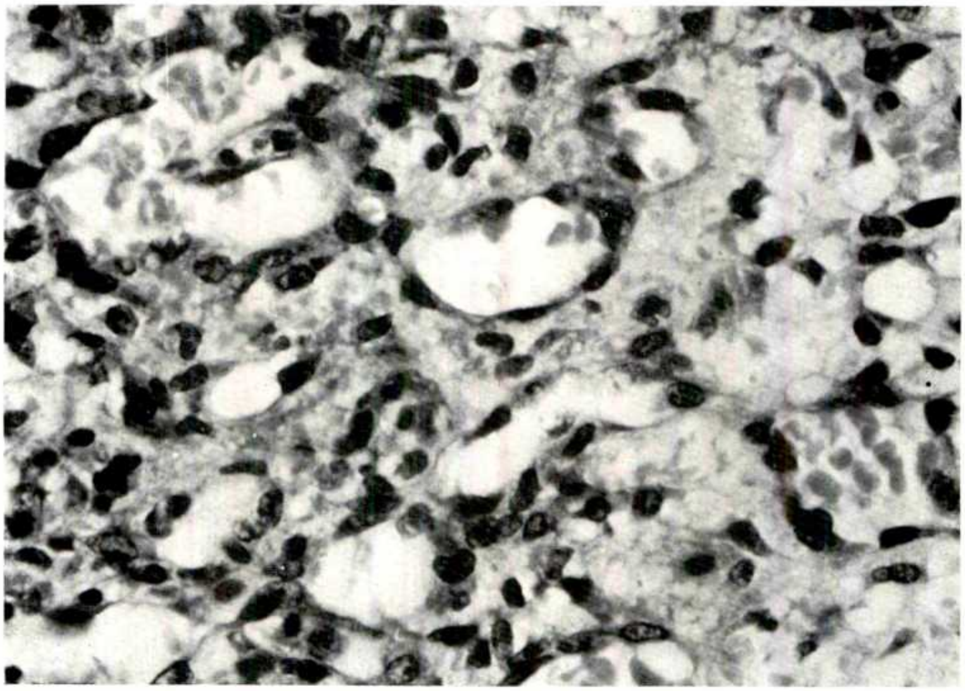

FIGURE 4. Case 3, high power photomicrograph showing numerous newly-formed capillaries lined by prominent endothelial cells and interspersed with lymphocytes and fibroblasts (H & E, × 510).

The clinical features and the histological findings, including the lack of the eosinophilic and PAS-positive intracytoplasmic inclusions, usually seen in the vacuolated epidermal cells of orf (Leavell *et al.*, 1968), as well as the absence of the viral particles of orf visible with the electron microscope (Nagington & Horne, 1962), exclude orf as a likely possibility.

DISCUSSION

Granuloma pyogenicum has long remained something of an enigma as regards its pathology and classification (McGeoch, 1961). It seems to occupy a no-man's land between a true neoplasm and a hyperplastic or reparative reaction (Warner & Wilson-Jones, 1968). Although some authors seem to favour a neoplastic nature (Knoth & Ehlers, 1962; Bhaskar & Jacoway, 1966), most workers would agree with its description as an over-zealous healing process producing exuberant granulation tissue with marked endothelial proliferation (Kerr, 1951).

The aetiology of granuloma pyogenicum is equally mysterious. Trauma, infection and hormonal influences have been put forward as causative factors. The hormonal factor is suggested by the observation that an oral lesion of this type, granuloma gravidarum, occurs in pregnant women. The infectious theory was strongly supported by early writers. Poncet & Dor (1897) described a lesion in man comparable to that described earlier, in horses, by Bollinger in 1870 (cited by Kerr, 1951), and designated it as 'human botryomycosis', implicating a fungal aetiology. Bodin (1902) suggested that the organism of botryomycosis was *Staphylococcus aureus* rather than a fungus. Recent writers have tended to dismiss infection as a factor. Bhaskar & Jacoway (1966) believed that the infectious theory had little to support it and suggested trauma as the most acceptable aetiological factor. Kerr (1951) suggested that granuloma pyogenicum results from some minor trauma which provides a pathway for invasion of micro-organisms and concluded that the lesion is of a complex aetiology and that it constitutes a response to injury of a particular type produced or influenced by some intrinsic factor.

In our cases, it is evident that the lesions are a direct result of the preceding vaccination. One or more of a variety of factors may be involved, including trauma, infection or an immunological reaction. It is difficult to understand how in vaccination, trauma alone can be the causative factor, as millions of people all over the world have been vaccinated without apparently developing this complication. The 'epidemic' occurrence of this lesion, after this vaccination campaign in Jordan, using the same batch of vaccine, inevitably raises the question whether the cause of this 'outbreak' lies in the nature of this particular batch of vaccine. It is to be noted that the constituents of this particular batch and the method of its application were identical with those previously used when this complication was not encountered. One likely variable, however, may be the inadvertent introduction into the vaccine of an unidentified microbiological agent, possibly a virus. This micro-organism may play the role of a secondary stimulus combined with that of trauma, as suggested by Kerr (1951), to initiate in a constitutionally susceptible subject a vasoformative reaction of the variety seen in granuloma pyogenicum. In support of the infective theory, one may speculate that the occurrence of multiple satellites in pyogenic granuloma (Warner & Wilson-Jones, 1968) may be infective in nature and not metastatic. Other clinical features encountered in granuloma pyogenicum, such as recurrence and possibly involution, may also be explained on this basis.

Whatever may be the exact aetiology and pathogenesis of the lesions in our cases, it is evident from our observations that granuloma pyogenicum can occur as a complication of smallpox vaccination. This new observation, we believe, adds support to the concept that more than one factor plays a role in the aetiology of this lesion, and the possibility of an infective, possibly viral, agent being one of these factors deserves further consideration.

ACKNOWLEDGMENTS

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A dermatosis specifically associated with a tumour of pancreatic alpha cells

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SUMMARY

On the basis of a detailed case report it is suggested that a distinctive dermatosis is specifically associated with a tumour of pancreatic alpha cells.

The story will be told of a lady in her fifties with an intractable remittent chronic superficial dermatosis with very distinctive characteristics, which nevertheless cannot be assigned any traditional diagnosis. Similar cases have been reported in association with pancreatic tumours and, as a result of discussions with colleagues, and after a number of preliminary investigations, it was decided to explore this lady's pancreas. At operation a pancreatic tumour was removed. No metastases were found. Her dermatosis recovered completely within a very few days and the severe diabetic state, which had occurred shortly before operation, ceased. It will therefore be suggested that for the first time it has been established that a specific skin change led to the pre-operative diagnosis of a specific internal tumour, removal of which immediately resulted in the disappearance of the dermatosis.

CASE REPORT

Mrs M. A. P. was born in 1919, and was just 50 years old when she first complained of irritation in the perineum and natal cleft. Hitherto she had always enjoyed excellent health, apart from some minor gynaecological disorders during her forties. There was no significant family history, and in particular no family history of diabetes mellitus. No glycosuria was found on several occasions during 1969, but it was found on one occasion in 1970. *Candida albicans* was grown from a vaginal swab in 1969 and again in 1970. A glucose tolerance test, carried out in 1970, was pronounced normal, but as this did reveal a degree of renal glycosuria the patient was advised to avoid a large intake of carbohydrate at any one time. Treatment of the candidal infection was apparently successful for a while, but her irritation, this time accompanied by a definite rash throughout the perineum, recurred early in 1971 and was soon followed by the appearance on her shins and fingers of redness and scaliness which proved difficult to control.

She was first referred to the author by Dr R. I. Maitland in October 1971, when it was considered to be the clinical picture of a candidal intertigo of the type associated with diabetes. Again, however, there was no convincing evidence of diabetes, and her lesions responded to topical amphotericin B and steroid applications. The perineal lesions did not completely clear, and though the hands and shins did so the trouble recurred there a few months later and became progressively worse.

There followed a period of extreme domestic upheaval and anxiety and, when seen again at the end of 1972, the patient had lost weight, appeared clinically anaemic and had an extensive intertriginous eruption extending through the groins and perineum and between the buttocks, and also a curious exfoliating eruption involving the whole of each palm and extending on to the dorsum of each of her fingers. There were scattered, rather nondescript, brownish-red papular lesions down the front of each lower limb from the knee to the dorsa of the feet, and similar lesions around her elbows. She complained that her tongue was uncomfortable and had been so for over a year. The tongue was smooth, shiny and a curious vermilion colour (Plate 1). As there was no immediate improvement with symptomatic treatment she was admitted to hospital. It was immediately found that she had in addition to a marked glycosuria, a blood glucose at 230 mg/100 ml. A year before it had been 85 mg/100 ml. It was thus thought that she had after all developed diabetes mellitus under our none too watchful eyes. Her haemoglobin was 11·5 g/100 ml; the blood picture otherwise was normal. Her ESR was 18 mm in 1 h. Swabs from the perineal lesions grew *Staphyloccocus aureus*, sensitive to all antibiotics against which it was tested. After an unrewarding week on chlorpropamide she was given soluble insulin 40 units daily. This initially controlled her diabetic state satisfactorily. Ketonuria was not found on any occasion during her illness.

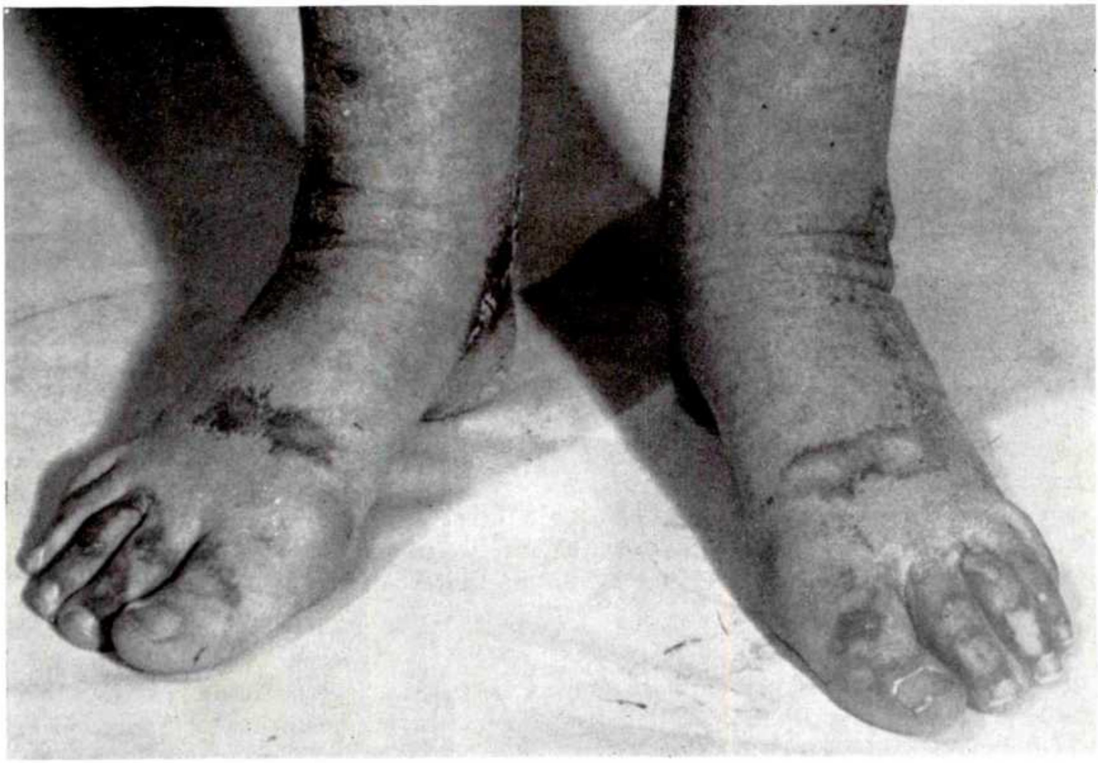

FIGURE 1. Erythemato-bullous lesions apparently determined by pressure of slippers.

There was, however, absolutely no improvement of her skin lesions. Also, she complained more of the discomfort of her tongue, and there was a marked loss of appetite. After the initial fall, her blood sugar remained over 200 mg/100 ml, in spite of progressive increases in the amount of insulin

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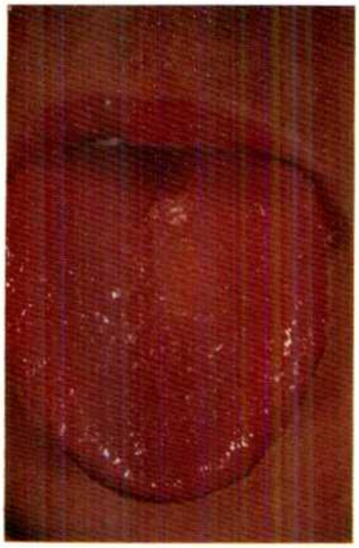

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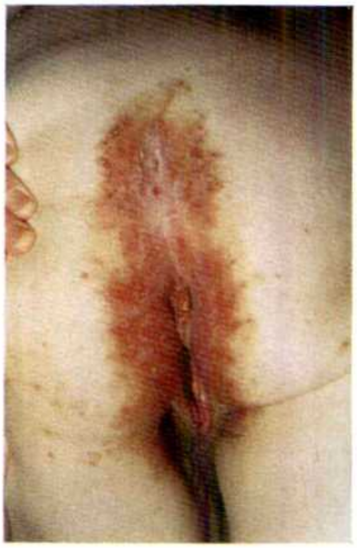

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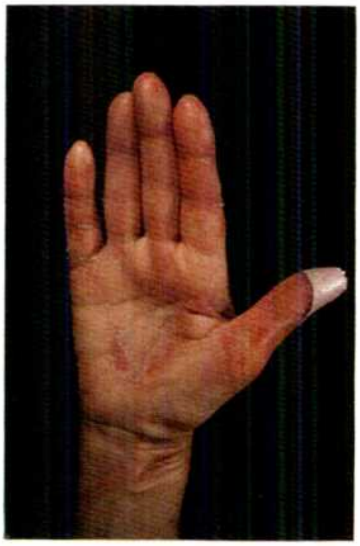

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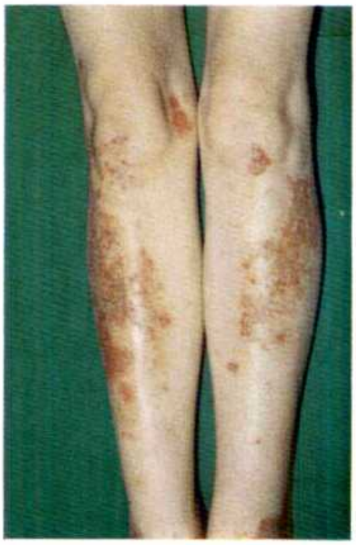

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given. Her skin lesions increased in extent and in severity. New lesions appeared on the backs of the thighs and in a band-like fashion across the dorsum of each foot, as though induced by contact with the upper part of a slipper. Within a short time most of her toes and large areas on the soles and around the ankles became affected. Her palms, which had previously improved, became more severely involved and in all these areas there was a tendency to very superficial vesicle formation, the vesicles seeming to coalesce to produce a spongy effect. There was also superficial vesicle formation at the edges of the intertriginous lesions in the groins and natal cleft, somewhat suggestive of pemphigus vegetans, apart from a rather coppery colour (Plates 2–4 and Fig. 1) A biopsy taken from this area showed oedematous parakeratotic disintegration of the upper half of the epidermis without acantholysis, quite unlike pemphigus.

The banded lesions on the feet and ankles momentarily suggested an allergic contact dermatitis, and patch tests were carried out against a standard battery of test substances and against material from the slippers she had been wearing in hospital. All these tests were negative, but the plaster strip which secured test pads to the skin of her back left an imprint darkish-red in colour but with no apparent inflammatory element to it; it persisted until her operation, and was quite unlike an ordinary plaster reaction (Fig. 2). A biopsy from this imprint showed minute areas of superficial spongiosis and parakeratosis. Biopsies from the vesicular lesions on her feet showed the same quality of change but much more marked oedema in and between squamous cells, disintegration of these cells and absence of keratin formation. Again the changes were superficial, and there was only a minimal cellular reaction in the dermis.

A month after admission the blood glucose level was 265 mg/100 ml, in spite of 56 units of soluble insulin daily, and 10 days later it was 285 mg/100 ml, in spite of 64 units a day. Her haemoglobin remained at 11.4 g/100 ml, uninfluenced by iron by mouth. Serum vitamin B_{12} level was normal on two occasions, and serum folate also was within the normal range.

A tentative diagnosis of pancreatic tumour, responsible both for her dermatosis and her diabetic state was made and she was transferred to the care of Dr C. N. Mallinson at the Greenwich District Hospital for further investigations. These investigations, which will be detailed elsewhere, showed that pancreatic secretion was markedly reduced both in volume and in bicarbonate concentration. Malignant cells were identified in the duodenal juice.

On her return to Plymouth, and after normal cholecystography and barium radiological studies of the duodenum suggested that the head of the pancreas was not affected, a sub-total pancreatectomy, including removal of the spleen, was carried out by Mr Michael Reece on the 6 March 1973. Within the pancreas, at about the junction of the head with the neck, was a spherical tumour 4 cm in diameter. No satellite lesions, enlarged lymph glands nor anything to suggest associated lesions in the liver or elsewhere in the abdomen were observed. The patient made a very satisfactory postoperative recovery, apart from some temporary leakage of pancreatic excretion through a drain incision. This was accompanied by a sharp but temporary rise in the serum amylase level which had previously been normal.

For the first week after operation, insulin was given by adding it to a continuous intravenous infusion of dextrose and normal saline according to requirements estimated by frequent blood sugar estimations. The daily dose fell from 34 units on the first day to 20 units on the sixth day. There was no glycosuria after the second day. Further progressive reductions were possible when intravenous infusions were stopped, without the blood glucose rising above 150 mg/100 ml, and on the tenth post-operative day Glibenclamide orally was substituted for insulin. Two weeks later this was stopped and a normal blood sugar was maintained on a slightly restricted carbohydrate diet alone, though a 100 g glucose tolerance test still showed a diabetic response. In the month before the operation she had lost 2·8 kg in weight. In the month afterwards she regained this weight, and her haemoglobin rose to 12.9 g/100 ml.

Her skin lesions showed a marked change by the second post-operative day. Generalized pink morbilliform lesions which had appeared a few days before operation had disappeared, as had the 'imprint' lesions on her back. All other lesions were becoming fainter and vesicles were drying and beginning to desquamate. Her tongue was losing its lurid red colour, and in another couple of days had returned to normal. By then her skin was no longer uncomfortable anywhere and apart, from some peeling, her dermatosis had ceased. Even her toes, which appeared quite raw before operation, had

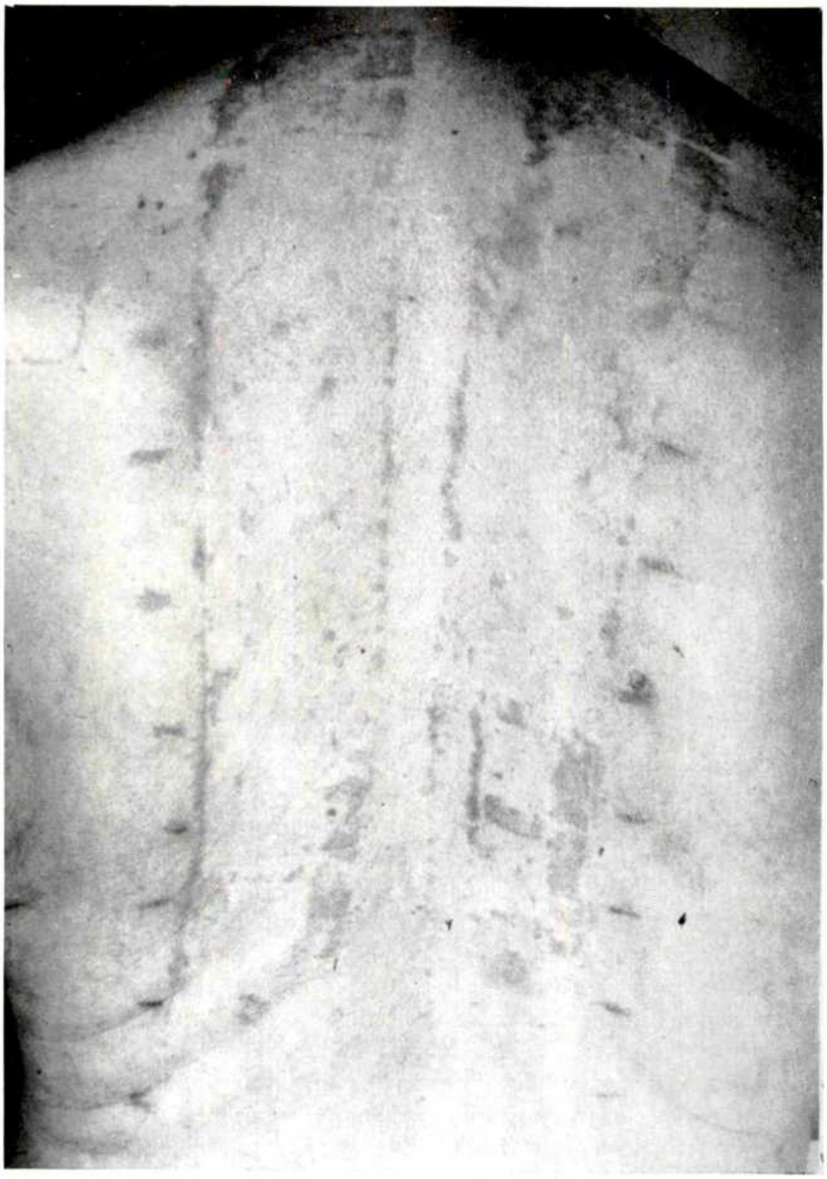

FIGURE 2. Fixed erythematous lesions produced by zinc oxide adhesive plaster used to secure patch tests (the lateral columns of markings are dye markers).

returned to normal in a week. Her palms had likewise recovered, but the soles, which had shown confluent bullae, did require some 3 weeks to reform a normal surface.

In the next 2 months she continued to gain weight. There was no glycosuria on a normal full diet and a 100 g glucose tolerance test repeated 3 months after her operation was normal. Her skin remained clear, without scarring and without the hyperpigmentation that at first marked the site of previous lesions.

HISTOLOGY

The skin lesions

The first biopsy was taken from the vesicular edge of the eruption in the groin and showed a moderate acanthosis accompanied by a relatively sparse lymphocytic infiltration and vascular dilatation confined to the most superficial part of the dermis. The basal cell layer and deeper epidermal cells were normal apart from small foci of intercellular oedema and the presence of occasional pyknotic polymorphonuclear leukocytes. Nearer the surface the intercellular oedema became progressively more marked and more and more individual squamous cells became swollen, their nuclei becoming smaller and more darkly stained. There was no granular layer. In some areas these swollen squamous cells merged into more tightly packed but still 'wet' looking parakeratotic cells; in others the intercellular oedema was so

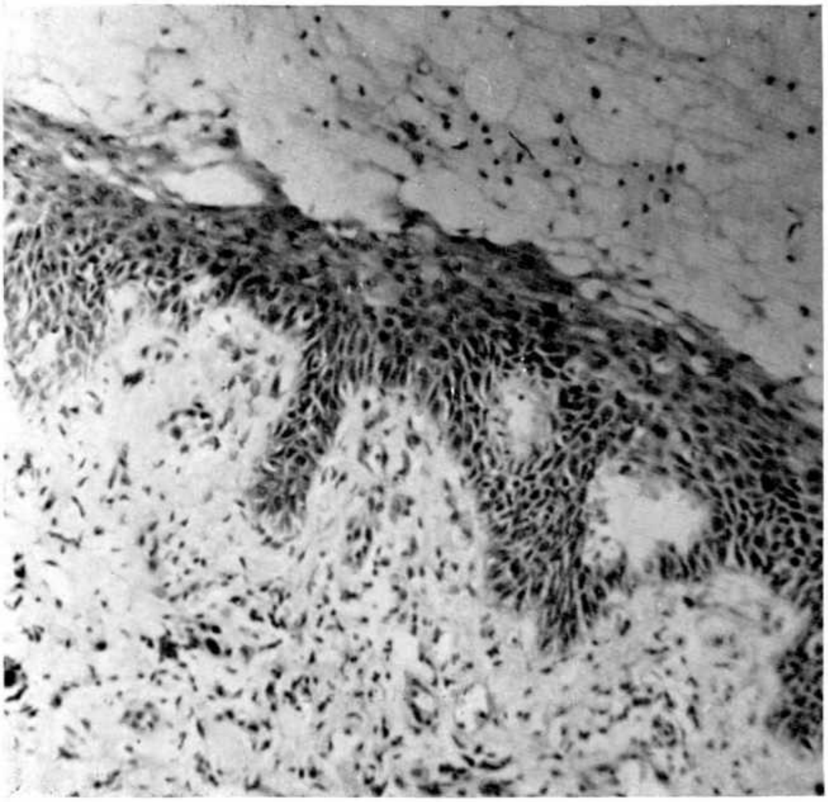

FIGURE 3. Bulla on dorsum of foot (PAS and Alcian Blue, × 95).

great that small vesicles were present with a very thin roof of apparently ill-formed keratin; in yet other areas the spongiosis appeared to extend to the surface.

Biopsies were taken later from the 'imprint' area on the back, from a recently erythematous area on one ankle and from a bullous area immediately adjacent. All showed changes of the same quality, though greatly varying in magnitude, except for the absence of acanthosis. On the back, where changes were focal, the epidermis seemed thinner than usual. Perhaps for this reason the intercellular oedema was not so obviously confined to the more superficial cells. It nowhere reached the extent of vesicle formation, and the foci were capped with small islands of parakeratosis. By contrast, the thicker epidermis on the side of the foot showed a normal basal layer and one or two tiers of normal squamous cells above; nearer the surface the cells became grossly swollen and more and more widely separated. These in turn were replaced by interweaving strands of faintly pink-staining material, like the fibres of a sponge. The interstices contained some scattered nuclear remnants but there was no proper parakeratosis. PAS and Alcian Blue preparation showed no mucin (Fig. 3). Sections from the erythematous area beside the bulla showed intermediate changes, again confined to the more superficial part of the epidermis (Fig. 4). In all biopsies the dermal changes were superficial and relatively insignificant apart from vasodilatation and oedema. In some areas, among the few lymphocytes present there seemed to be a disproportionate number of hystiocytes and fibroblasts.

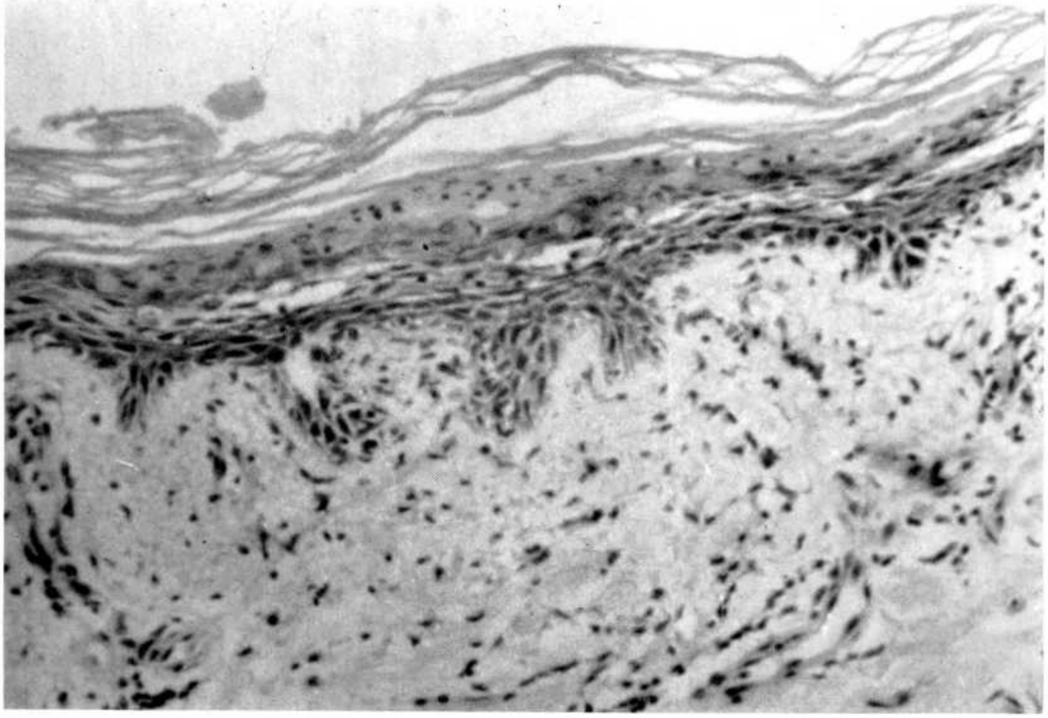

FIGURE 4. Raised erythematous area on dorsum of foot (H & E, × 95).

The pathological state revealed by these sections, in various degrees of severity, would seem to be a perfusion of fluid into the epidermis accompanied by swelling and premature death of the cells as they near the surface and before they have an opportunity to ripen into keratin.

The term superficial epidermal necrolysis can well be applied to such a process.

The tumour in the pancreas

Dr A. C. Hunt reported as follows: 'The tumour is made up by sheets and trabeculae of pleomorphic cells, with large rather open nuclei mostly with a single large nucleolus. There are a fair number of hyperchromatic cells, but not many mitoses. Scattered in the tumour are well defined tubular structures. The edges are ill defined, and small groups of cells are invading surrounding pancreas. although no tumour can be seen in lymphatic or vascular channels. The appearances are those of malignant islet cell tumour.'

It proved impossible to determine, by demonstration of the typical granules, whether the tumour cells were α or β islet cells. Subsequent electronmicroscopy showed that the numerous granules present were spherical, homogeneous, opaque and sharply outlined, as found in α cells. β cell granules are more varied in size and shape, often crystalline and usually surrounded by a clear halo (McGavran *et al.*, 1966).

Additional investigations

Glucagon assay. Dr C. R. Bloom reported that the level of pancreatic glucagon in fasting serum collected before operation and measured by specific radioimmuno-assay was 3500 pg/ml. 1 week after operation it was 130 pg/ml (normal range 40–120 pg/ml). The tumour was extracted by the method of Kenny (1955) and was found to contain 4 μg/g wet weight.

Plasma protein electrophoresis. There was no discernible difference between parallel strips from pre- and post-operative sera. There was slight elevation of IgM and IgA in each.

Skin reactions. A week after operation 0.1 ml of normal saline, pre-operative and post-operative serum and of a commercial glucagon preparation were injected intradermally, each at two sites, on one forearm. One set was subjected to the pressure of a tight bandage. There was no reaction at any injection site.

DISCUSSION

There is only one thing unique about this case; successful removal of her pancreatic tumour resulted in the recovery of her dermatosis and the recovery of her diabetes mellitus. Yet Becker, Kahn & Rothman (1942), in a beautiful clinical description of a female patient aged 45 who died of an islet cell carcinoma of the pancreas, used the word 'unique' to describe her dermatosis. This description enabled Church & Crane (1967) to be sure that their female patient aged 61, who died of an islet cell carcinoma of the pancreas with a dermatosis with highly specific characteristics, was similar in every respect. Both patients had been mild diabetics. McGavran *et al.* (1966) reported a female diabetic patient aged 42 in whom investigation of hepatomegaly led to the diagnosis of an islet cell carcinoma in the tail of the pancreas which was later removed, though this was not possible for the liver metastases. This patient had a persistent rash affecting the hands, feet and perineum which 'defied specific diagnosis'. Her tumour was shown to be a malignant growth of α cells and to contain large amounts of glucagon, which was considered responsible for her diabetic state. Wilkinson (1971) recalled Church & Crane's case when he presented yet another mild diabetic female, aged 55, with a rash so similar that he suggested it must be diagnostic of a malignant growth of the pancreas. Such a growth was found on laparotomy, again with secondary deposits in the liver. He suggested the name 'necrolytic migratory erythema' for these remittent chronic circinate desquamating lesions with pseudo-bulla formation affecting particularly the groins, perineum and limbs. The histology of the skin lesions was the same as in Becker's and in Church & Crane's cases.

It must be emphasized that it was entirely as a result of the above observations that the case reported here was diagnosed, operated upon and apparently cured. Her skin changes were so similar that there seems no doubt that this appearance of very superficial burns with attempts at blistering, affecting particularly areas where skin is submitted to friction, is a truly separate condition, identifiable clinically and histologically. There seems equally little doubt that this clinically identifiable dermatosis is related to and indeed caused by some pathological state of a particular internal organ, namely the pancreas. Such a degree of specificity of cause has not been recognized with other organs, and the pancreatic lesion is usually, if not always, an islet cell carcinoma. Possibly it is always a glucagonoma. Removal of such a tumour can relieve the dermatosis and save the patient.

The mechanism by which such a tumour, or perhaps some secondary destructive effect it may have on part of the pancreas, can induce a specific and apparently purely epidermal skin change is as yet unknown. A more comprehensive study, embracing endocrine and gastro-enterological aspects of glucagon-producing tumours, is planned with colleagues who have helped so much in the investigation of this and pathologically related cases. However, some other features common to the cases mentioned above are worth noting. The skin lesions in each ran a very similar chronic course of about 3 years; in each a curious, uncomfortable, red, shiny, raw-looking tongue was a feature; all lost weight, whether or not as a result of their cancer, and all had a refractory normochromic anaemia which seemed not to be a result of malabsorption. One particular aspect of the Plymouth case, whose rash reached a greater pitch than any of the others, was the determining effect of friction or pressure upon the sites at which lesions appeared.

CONCLUSION

It is justifiable and indeed important to classify cases such as the one reported in this paper as having a 'Pancreatic dermatosis'. The triad of a chronic superficial necrolytic dermatosis plus a smooth shiny vermilion tongue plus hyperglycaemia is the strongest indication for operation with the hope and intention of removing a tumour from the pancreas.

ACKNOWLEDGMENTS

Dr C. N. Mallinson swung the balance by his investigations of pancreatic function. Mr Michael Reece skillfully cured the patient; his house surgeon Dr S. L. Lightman who managed the post-operative recovery and diabetic transition told me all about glucagon. Dr S. R. Bloom carried out the glucagon assays. Dr A. C. Hunt and Dr M. R. Thomas instructed me on the histological appearances and Miss Sylvia Easton took the photographs. Dr Lightman has already reported the diabetic aspects of the case. We are all indebted to the Eli Lilly Company for the cost of the colour plates.

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Blood and pustule culture in pustular psoriasis

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SUMMARY

Blood and pustule cultures from patients with generalized pustular psoriasis showed no growth of bacteria or of mycoplasmas. Similar negative results were obtained from patients with pustular psoriasis confined to palms and soles. The significance of some of these results is discussed.

In 1971, McFadyen & Lyell reported that coagulase positive staphylococci could be isolated from the blood of seven patients suffering from generalized pustular psoriasis (von Zumbusch type). This prompted us to look again at some of our cases with generalized pustular psoriasis from this point of view despite previous negative results. At the same time, we looked for the presence of mycoplasmas in the blood and in the pustular lesions of these same patients. Some cases of pustular psoriasis localized to the palms and soles were also included in this study.

MATERIALS AND METHODS

Routine bacteriological investigation

Eight patients with generalized pustular psoriasis and three patients with pustular psoriasis of palms and soles were studied. Cultures from the blood and from the pustular lesions were taken on admission as well as during the hospital stay, particularly during episodes of pyrexia exceeding 38°C which some of these patients developed. In several of these patients who had no rise in temperature and yet showed a flare-up of their lesions, blood cultures were taken as soon as the flare up was clinically evident. The venepuncture site was swabbed for culture each time blood was taken. The blood culture media consisted of plain broth, broth with 0.5% bile salt, and digest broth with 0.1% glucose (Southern Group Laboratory). The blood cultures were incubated at 37°C and subcultures were made on three consecutive days and one week later. Pus from the skin lesions was obtained on a swab after cleaning the surface of the pustules with 70% isopropyl alcohol, then lancing them with a sterile needle. All swabs were plated on blood agar under aerobic and anaerobic conditions as well as on MacConkey, chocolate and CLED media.

Investigation for mycoplasmas

Six patients with generalized pustular psoriasis and six patients with pustular psoriasis of palms and soles were studied. Blood for culture was taken at the same time as for the above investigation and

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TABLE 1. Details of cases with generalized pustular psoriasis, all presenting with typical skin lesions

Case no.	Sex	Age (years)	Pyrexia and other symptoms*	Blood cultures		Pustules cultured		Treatment
				No. samples	No. positive	No. samples	No. positive	
1	F	6	Pyrexia Malaise	5	0	None		No response to cephaloridin and cloxacillin. Good response later to MTX†
2	F	75	Pyrexia Malaise	2	0	None		Good response to MTX
3	F	27	No pyrexia Malaise	1	0	1	0	Topical treatment
			Pyrexia Malaise	1	0	2	0	Started on MTX. Good response
4	F	50	Psoriatic arthropathy					
			No pyrexia	2	0	2	0	
			Pyrexia and malaise	1	Gram +, coagulase negative coccus	1	0	Good response to MTX, which was given after the blood culture was taken
5	M	40	Pyrexia and malaise	1	0	1	0	Good response to MTX
			No pyrexia	1	0	1	0	Good response to MTX
6	F	27	No pyrexia	2	0	2	0	Good response to MTX
7	F	28	No pyrexia	1	0	1	0	Good response to MTX
8	M	67	No pyrexia	1	0	1	0	Good response to MTX

* At the time of investigation. † MTX, methotrexate.

was placed in PPLO broth (Difco) supplemented with 10% fresh yeast extract and 20% unheated horse serum. The blood cultures were incubated at 37°C, and subcultures were made 2 and 7 days later onto PPLO agar, supplemented by the same concentration of yeast extract and horse serum. The plates were then incubated anaerobically at 37°C in a mixture of 95% nitrogen and 5% CO_2, and checked for growth after 3 and 7 days. Cultures from the pustules were taken as swabs that were put immediately into a transport medium consisting of PPLO broth, 10% yeast extract and 20% horse serum. Penicillin (1000 units/ml) and thallium acetate (a final concentration of 1/2000) were added as bacterial inhibitors. These swabs were later checked for mycoplasmas using the colour-change indicator technique (Taylor-Robinson & Purcell, 1966). Each batch of horse serum was checked for any possible antimycoplasmal activity, by running a positive control with known cultures of mycoplasmas.

RESULTS

Routine bacteriological investigation

No organism was isolated from the blood cultures of the eight patients with generalized pustular psoriasis except on one occasion: a gram positive, coagulase negative coccus was grown from the three blood culture bottles in case 4. This coccus, however, had the same sensitivity pattern and the same morphological characteristics as one isolated from the venepuncture site at that time. Although typing was not carried out, this organism was considered to be a contaminant. Negative results were also obtained from the patients with pustular psoriasis of palms and soles. More details are given in Tables 1 and 2.

TABLE 2. Cases with pustular psoriasis of the palms and soles, without any systemic manifestation

Case no.	Sex	Age (years)	Blood cultures		Pustules cultured	
			No. samples	No. positive	No. samples	No. positive
1	F	72	10	0	12	0
2	F	72	5	0	5	0
3	F	20	1	0	2	0

Investigation for mycoplasmas

No mycoplasmas were isolated from the blood or from the pustular lesions of either group of patients.

DISCUSSION

In all eight cases of generalized pustular psoriasis, no organisms could be isolated either from the blood or from the skin lesions. Cases 1 and 2 showed typical widespread pustular skin lesions associated with high fever, malaise and leukocytosis. Cases 3, 4 and 5 also showed widespread skin lesions and on one occasion only, each had systemic symptoms and pyrexia. The rest of the patients showed typical widespread skin lesions with minimal systemic manifestations and no rise in temperature. These patients were already receiving treatment with methotrexate (MTX) and were in hospital for re-evaluation. Case 1 was given a trial of systemic antibiotics for several days without any response. As her clinical state proved to be resistant also to topical therapy, she was given MTX to which she responded. The remaining cases also showed a good response to MTX therapy. The patients with psoriasis of the palms and soles were receiving only topical treatment.

MTX inhibits certain bacteria, such as *Lactobacillus casei* and *Streptococcus faecalis*, by interfering with the folic acid metabolism (Franklin *et al.*, 1947; Franklin *et al.*, 1949; Delmonte & Jukes, 1962). Metcalfe & Hughes (1972) have demonstrated that the incidence of group A beta haemolytic streptococci is much lower in leukaemic children receiving MTX therapy than in a control group and an *in vitro* experiment proved that MTX is inhibitory to this streptococcus. However, in the same paper, these authors demonstrated that other bacteria are not inhibited by MTX; indeed one, *Staphylococcus aureus*, was shown to be resistant to MTX even at a concentration of 250 μg/ml. It is therefore difficult to believe that a temporary bacteraemia, particularly with *S. aureus*, can occur asymptomatically in patients receiving MTX. In our patients, there was a good response to MTX therapy with no evidence of sepsis.

In this paper, the investigation on mycoplasmas was a preliminary one based only on cultures and, at this stage, a negative finding has to be interpreted cautiously. Negative results from cultures do not necessarily mean that mycoplasmas are not present, as there is still need to establish optimal conditions, media and culture procedures for their isolation and growth (Hayflick, 1972). Also, the possibility of their playing a role early in the onset of the disease or in a different region of the body cannot be eliminated.

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Reduced activation of epidermal phosphorylase in the lesions of psoriasis

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SUMMARY

The percentage activation of the enzyme phosphorylase in extracts prepared from the epidermis of psoriatic lesions was found to be significantly lower than normal. This observation lends strong support to the concept of a reduced level of cyclic AMP in the lesion of this disease.

An elevation of the mitotic rate and an accumulation of glycogen are consistently observed in the epidermis of psoriatic lesions. It has recently been suggested (Voorhees & Duell, 1971) that both of these phenomena may be secondary to a reduced level of epidermal adenosine 3′5′-cyclic monophosphate (cAMP). Attempts to test this hypothesis directly, however, have led to contradictory findings; thus Voorhees *et al.* (1973) reported a reduction of 22% in the cAMP level of psoriatic epidermis, whereas Hopsu-Havu, Harkonen & Raij (1973) found cAMP to be 'slightly elevated'. Measurements of the related enzymes are also conflicting. Wright *et al.* (1973), for example, claimed a substantial reduction in adenyl cyclase activity; Hopsu-Havu *et al.* (1973), on the other hand, reported little change in adenyl cyclase activity but a striking reduction in the phosphodiesterase level in the psoriatic lesion.

It is clear that independent evidence concerning the cAMP level in psoriatic epidermis would be of value. It is now well documented that the glycogenolytic effect of cAMP is mediated by the sequential activation of the enzymes cAMP-dependent protein kinase, phosphorylase kinase, and phosphorylase. In consequence, any change in the intracellular cAMP level will be manifested by a corresponding alteration in the fraction of phosphorylase existing in the active form (phosphorylase *a*). Since sensitive and specific methods are now available for the measurement both of phosphorylase *a* and of total phosphorylase (Mier & Sutorius, 1972), it seemed of interest to determine their ratio in normal and in psoriatic epidermis. Our results are presented in the following communication.

MATERIALS AND METHODS

Reagents were in general of analytical grade; coupling enzymes were purchased from the Sigma Chemical Co. Samples of epidermis (about 0.5 cm^2) were taken from the upper arm of healthy subjects (nine), from the 'uninvolved' skin of the upper arm of psoriatic patients (nine), or from active psoriatic lesions (thirteen). All specimens were cut using a Castroviejo keratotome with the blade set at 0·2 mm depth; no anaesthetic agent was employed. The samples were dropped into liquid nitrogen within a few seconds of their removal, and were kept under liquid nitrogen until homogenization. Immediately prior to assay, the epidermis was transferred to an homogenizer vessel ('Micro-wet grinder', Townson and Mercer) containing 2 ml of ice-cold buffer (0·02 mol/l phosphate, 2×10^{-4} mol/l EDTA and 10^{-3} mol/l $MgCl_2$ at pH 6.7), homogenized quickly and centrifuged.

The phosphorylase activity of the extract (usually 0·5 ml aliquots) was assayed in the direction of glycogen breakdown; glucose-1-phosphate was converted to glucose-6-phosphate with phosphoglucomutase, and the latter measured by NADP coupled oxidation in the presence of glucose-6-phosphate dehydrogenase. Phosphorylase *a* activity was calculated from the initial reaction velocity, and the total phosphorylase activity from the reaction velocity recorded after the addition of 5′ AMP (2 mmol/l final concentration) to the cuvette. Details of the procedure were similar to those described previously (Mier & Sutorius, 1972), except that in the present experiments NADPH synthesis was followed spectrophotometrically (Unicam SP 8000, absorbance range 0.2) rather than fluorimetrically. The 'percentage activation' of the phosphorylase was defined as: (initial reaction velocity/reaction velocity after addition of 5′ AMP) × 100.

RESULTS

Preliminary experiments showed the observed value for percentage activation to be reproducible (±5%) for duplicate aliquots of a single extract, and to be independent of the concentration of epidermis in the extract for reaction velocities (total phosphorylase) not exceeding about 0·01 OD units/min. The most serious potential error was associated with the interval between cutting the epidermis and freezing; the percentage activation of the phosphorylase rose to a figure approaching 100% within 2 min of biopsy if the specimen was kept at room temperature. However, relatively little change occurred after homogenization, the percentage activation of the phosphorylase falling by less than 10% after 1 h at 0° C.

TABLE 1. The percentage activation of phosphorylase in extracts of normal epidermis, the uninvolved epidermis of psoriatic patients, and the epidermis from psoriatic lesions

Nature of specimen	No. of specimens investigated	Mean value of percentage activation	Standard error of the mean
Normal epidermis	9	64·8	2·7
Psoriatic 'uninvolved' epidermis	9	66·1	2·6
Epidermis from psoriatic lesion	13	49·3	2·4

The mean values for the percentage activation of phosphorylase in extracts of normal epidermis, of the 'uninvolved' epidermis of psoriatic patients, and of the epidermis from psoriatic lesions are shown in Table 1. The difference between normal epidermis and the lesion is highly significant ($P < 0{\cdot}005$). Individual values are illustrated in Fig. 1; here the untreated lesions (six specimens) and those which had received some therapy prior to biopsy (seven specimens) are shown separately.

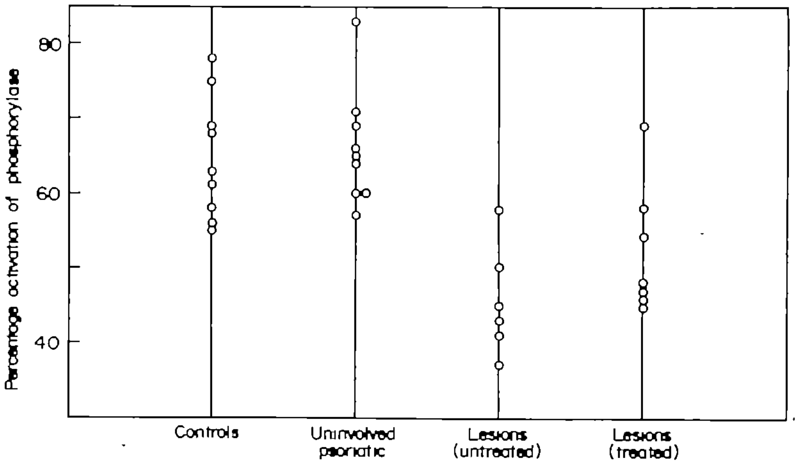

FIGURE 1. Percentage activation of phosphorylase in the individual extracts. The patients in the 'treated' group had received either topical applications or systemic methotrexate therapy for 1–3 days prior to biopsy, but were still clinically active.

DISCUSSION

It must be emphasized that the 'percentage activation', as measured by the present technique, is subject to certain limitations and does not necessarily represent an absolute estimate of the degree of activation of epidermal phosphorylase *in vivo*. The chief practical problem is the extremely rapid activation of the enzyme in response to biopsy, presumably a result of acute ischaemia. Our observations are similar to those of Kondo & Adachi (1971), who found that the activation of another cAMP-controlled enzyme (phosphofructokinase) was apparent within 5 s of plucking hair bulbs. A second difficulty is that epidermal phosphorylase *a* may itself be marginally stimulated by 5′ AMP, as is the case with the muscle enzyme. Although this seems unlikely from our present data, an absolute figure cannot be established until the behaviour of isolated epidermal phosphorylase *a* has been investigated.

Nevertheless, the highly significant difference between the epidermis from normal skin and that from the lesion must be regarded as strong support for the hypothesis of Voorhees & Duell (1971). Although factors other than cAMP could, in theory, influence the apparent 'percentage activation' of phosphorylase (for example the 5′ AMP content of the epidermal sample), these should certainly be diluted out under the conditions described. It is of interest that the 'uninvolved' epidermis shows a normal level of phosphorylase activation; this seems to rule out the possibility that a disturbance of cAMP metabolism is the primary congenital abnormality in the psoriatic individual.

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Pharmacology and Treatment

Combined 8-methoxypsoralen and black light therapy of psoriasis

TECHNIQUE AND RESULTS

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SUMMARY

Seventy-four patients with psoriasis underwent a combined topical treatment with an 8-methoxypsoralen solution and black light. In thirty-four patients the skin lesions healed; in twenty-three patients they improved considerably. Eleven patients showed only a moderate regression of the lesions, whilst in six patients treatment was for various reasons discontinued during the first phase. The 8-methoxypsoralen–black light therapy can be performed in out-patients and has the advantage over dithranol treatment of being entirely clean. Relapses are not resistant to further similar treatment.

For some years the treatment of discoid psoriasis with corticosteroids or cytostatic agents has been less often employed than formerly and it is now reserved for the severe forms. The high recurrence rate after corticosteroids and the danger of known side effects of the cytostatics may be the reasons. This situation has been followed by the increasing recourse to the classical local treatment with chrysarobin in the form of dithranol (1,8-dihydroxyanthranol) which had been recommended by Galewsky (1916) for psoriasis. Its therapeutic effect is acknowledged but its employment usually involves admission to hospital, and always has the disadvantages of serious soiling of skin and linen, of subjective discomfort and finally, the danger of skin irritation which may result in exacerbation of the psoriasis. These disadvantages are responsible for the continuing search for new and effective treatment, with minimum side effects. This will of course normally be only symptomatic treatment, as long as the aetiology of the psoriasis vulgaris remains unknown.

Since the summer of 1972, following the suggestion of Oberste-Lehn (1972), we have treated seventy-four patients with chronic psoriasis by the local application of 8-methoxypsoralen (8-MOP) and subsequent phototherapy with black light (Weber, 1973). The therapeutic results are so convincing and the application so simple that we were able to carry out this treatment as a routine and were able, with few exceptions, to abandon the controversial therapy with corticosteroids and cytostatic drugs. We used the dithranol treatment only in those cases in which the combined 8-MOP and black light treatment had failed. Even then it was observed that after this pretreatment the period of dithranol therapy could be curtailed.

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METHOD

A 0·15% solution of 8-MOP in isopropyl alcohol was brushed on the psoriasis lesions. After an interval of 1 h the phototherapy with black light (Table 1) was given in a chamber which we constructed ourselves (Fig. 1). This chamber* consists of a 2 m high cylinder which stands vertically, is movable and can be opened up on hinges (Fig. 2). The horizontal diameter of the chamber is 1·5 m. On its walls are evenly distributed a total of thirty tubes of the type Sylvania F 20 T 12-BLD black light blue. The maximum wavelength of the output of these lamps is 360 nm. The inner wall of the chamber is lined with aluminium foil to reflect the radiation. The cylindrical shape of the chamber results in equal radiation of the whole body surface. In contrast to a square or hexagonal chamber (Fig. 3) there are no beam deflections, interferences, potentiations or reductions of radiation intensity: thus the light energy applied to the skin is constant depending only on the duration of exposure. Dosage and therapeutic comparisons are therefore possible.

The 8-MOP solution† is free from amoidine. The peak absorption of the solution lies at 360 nm, which is in accordance with the emission maximum of the black light lamp which has already been referred to by Stegmair (1959).

CASE MATERIAL AND DURATION OF TREATMENT

Seventy-four patients, 15 to 62 years old, with a variety of clinical forms of psoriasis, were treated according to the scheme outlined in Table 1. The phototherapy started with a duration of 8 min, which was increased over 5 consecutive days to 12 min. However, psoriasis of the palms and soles was exposed for up to 25 min. According to these schemes, the light treatments were carried out on 5 consecutive days in both the first and second weeks with an interval of 2 days between the two courses. After the second week of treatment an interval of 7 days followed. Then treatment was resumed in the same 2 week pattern until healing occurred.

An inflammatory reaction in the form of an exacerbation and slight elevation of the psoriatic plaques could usually be observed after the third to fifth exposure to light. In all cases where 8-MOP had inadvertently been applied to the unaffected skin an erythema developed equivalent approximately to the erythema after 3 min UV radiation, with subsequent pigmentation as in Berloque dermatitis (Fig. 4). The involutional phase of the psoriatic lesion began after a variable number of exposures. It was first evident as a decrease in scaling of the lesions, which then flattened; the erythema faded. Brownish pigmentation replaced the psoriatic plaque.

Six patients with psoriasis, not included in the above case material, were treated exclusively with black light irradiation starting with a duration of 8 min increasing to 12 min over a total of 10 days. In these patients the clinical condition of the psoriasis did not change. Another four patients with psoriasis were treated on 10 consecutive days with 8-MOP only, the lesions being protected from light. In these cases too, no influence on the lesions could be detected.

DISCUSSION

The first combined topical application of 8-methoxypsoralen and black light for a dermatological

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* Manufacturer: Fa. Atmos, 7825 Lenzkirch, F.R. Germany.

† Meladinine®: Dr med. J. Ellendorff & Co., 56 Wuppertal, F.R. Germany.

TABLE 1. Scheme of the black light treatment of psoriasis

0·15% Ammoidin (8-methoxypsoralen)–isopropyl alcohol solution
  (1) Brush on to the descaled lesions
  (2) 1 h interval
Black light radiation:
  1st week: on 5 consecutive days
    increase duration from 8–12 min
  2nd week: on 5 consecutive days
    12 min duration each
  3rd week: no radiation
  4th week: as for 2nd week
  5th week: as for 2nd week

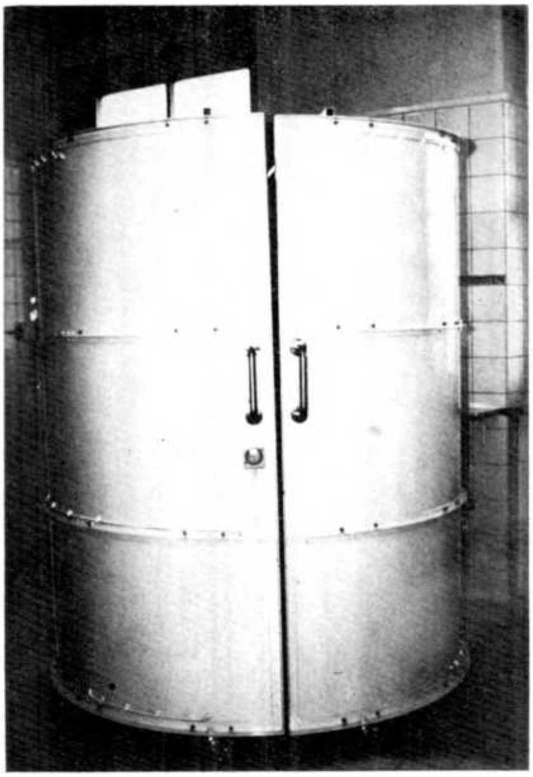

FIGURE 1. The closed black light chamber.

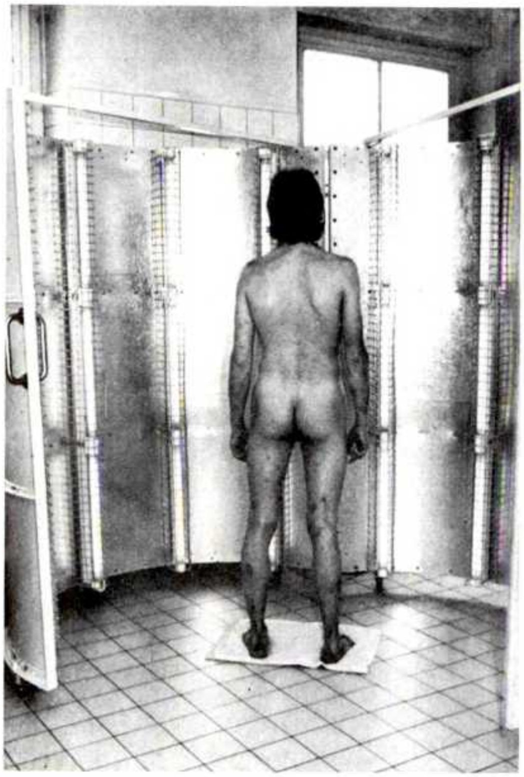

FIGURE 2. The opened chamber with black light beams.

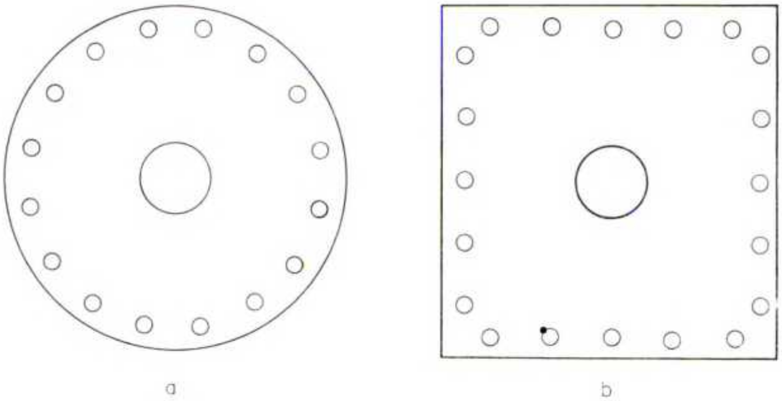

FIGURE 3. (a) Round chamber, no beam deflections are possible; in contrast to (b) square-shaped chamber.

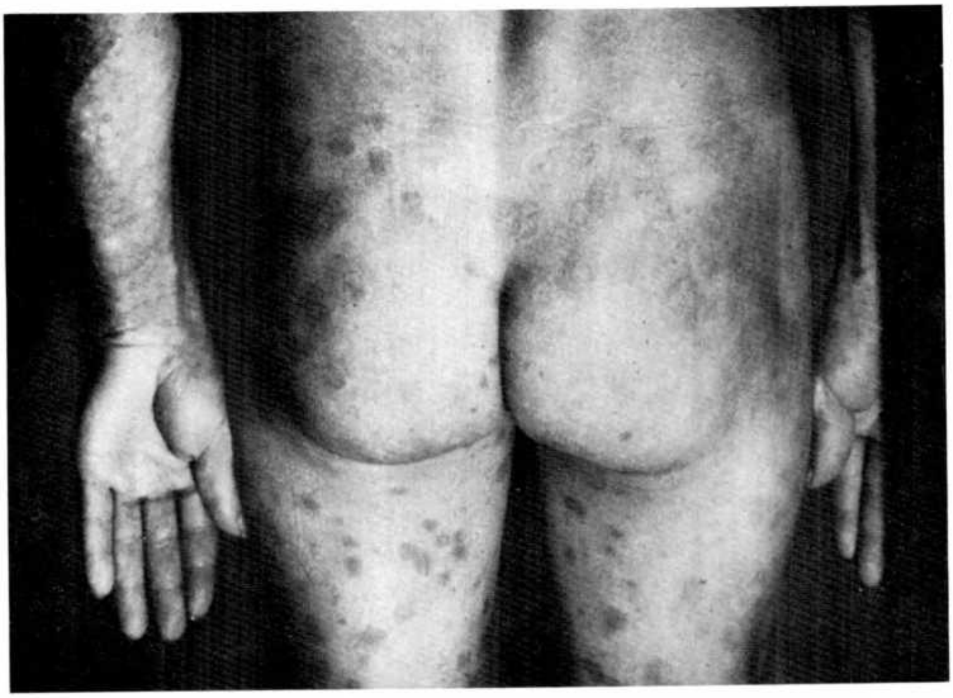

FIGURE 4. Berloque dermatitis-like pigmentation subsequent to 8-MOP–black light treatment.

condition was made in cases of vitiligo by Fulton, Leyden and Papa (1969). The authors applied 0.1% methoxsalen in a hydrophilic ointment to vitiligo lesions and then exposed these to radiation with black light, at a distance of 30–35 cm from the patient. In three of fifteen vitiligo patients complete re-pigmentation was observed; in six patients there was approximately 50% re-pigmentation. These authors preferred black light to short wavelength UV-light in order to avoid side effects, such as severe erythema, oedema and vesiculation.

In thirty-four of seventy-four patients with psoriasis vulgaris, which had been treated in the manner stated, healing of the skin lesions was observed after twenty to thirty-four exposures. In these patients no differences in the therapeutic effect on the various forms of psoriasis were noted, except for pustular psoriasis. In another twenty-three patients, the skin condition improved considerably after nineteen to forty-two exposures (Table 2). In this group the psoriatic lesions on the trunk and limbs were mostly healed, but in areas not easily accessible to light, i.e. in hairy areas like the scalp, and in the anogenital region, the psoriasis persisted.

For the acute state of psoriatic erythroderma, the duration of radiation should not exceed 8 min

TABLE 2. 8-MOP–black light therapy in seventy-four patients with psoriasis

34 patients healed after 20–34 exposures
23 patients greatly improved after 19–42 exposures
11 patients moderately improved after 8–26 exposures
6 patients not improved, treatment discontinued

during the first 10 days in order to avoid an exacerbation. In three patients with psoriatic erythroderma, i.e. generalized psoriasis, the 8-MOP was therefore applied alternately on different body regions to avoid exposure of the whole skin to the reaction at the same time. Since black light alone does not lead to a visible reaction on the skin, those body regions which have not been treated with 8-MOP need not be protected against radiation.

Apparently the 8-MOP solution diffuses quickly through the skin (Oberste-Lehn, 1972) since, after an interval of 8 h, radiation with black light no longer causes an erythematous reaction. Therefore, a cumulative effect of the black light in the event of repeated irradiation but only a single application of 8-MOP is not to be feared. However, if the patient is exposed to sunlight within 4–6 h after the application of 8-MOP, then intense erythema appears. We therefore exempted psoriatic lesions of the face from the treatment in order to avoid the possibility that daylight, i.e. sunlight, could lead to an uncontrollable potentiation of the black light treatment.

In consequence of the erythema an intense and more persistent pigmentation develops in the areas of application of 8-MOP. On the face, this is considered by the patient to be cosmetically unacceptable. For these reasons we also avoided systemic administration of 8-MOP, to avoid radiation reactions of the unaffected skin as well as a cumulative effect due to sunlight.

Psoriasis on palms and soles requires a longer period of irradiation. The reasons seem to be the slow diffusion of the 8-MOP solution and decreased penetration of the black light through the thicker horny layer. In this connection, it is emphasized that the 8-MOP and black light treatment is worth while only if the psoriasis lesions have previously been thoroughly freed from scale. This procedure has, therefore, to be repeated daily throughout treatment.

In eleven of the seventy-four psoriasis patients, the improvement after eight to twenty-six exposures was only fair, but we could find no reason for the less favourable response. It may be that the responsiveness of the psoriatic lesion was reduced by inflammatory changes, a phenomenon which is known with dithranol and ultraviolet light therapy. Whether more intense pigmentation of the skin before treatment began was a factor cannot be answered retrospectively.

In six patients, the treatment was discontinued when eight to ten exposures had produced no satisfactory response. Discontinuance of black light therapy was necessary since an exacerbation of the psoriasis occurred during treatment. One patient with pustular psoriasis showed fresh pustulation after the eleventh exposure. In two other patients with discoid psoriasis the possibility can not be excluded that a fresh eruption of the psoriasis was induced by an intercurrent acute febrile tonsillitis.

It must be assumed that in the remaining three patients exacerbation of the psoriasis occurred because they showed an increased sensitivity to the combined 8-MOP–black light treatment which induced a Koebner phenomenon, as is sometimes also observed in psoriasis after intensive exposure to sunlight.

When comparing these therapeutic results with the known effect of dithranol therapy, the 8-MOP–black light therapy does not show any advantage as far as the duration of treatment is concerned. In agreement with Mortazawi & Oberste-Lehn (1973), we ascertained that the 8-MOP–black light treatment, including the therapy-free intervals, lasts longer than in-patient treatment of psoriasis with dithranol, which requires on average about 20 days. But, the essential advantage of the combined treatment is that it can be carried out in out-patients with a therapeutic success rate about the same as that of dithranol therapy. Of essential importance to the patient is the fact that staining of the skin and soiling of the clothing and linen, which are unpleasant side effects of dithranol or tar and ointments, are avoided. It is further worthy of comment that the response of relapsing psoriasis to further 8-MOP–black light therapy is not diminished.

Theoretical support for the therapeutic effect of 8-MOP and black light is provided by a paper just published by Walter *et al.* (1973). These authors demonstrated that the DNA-synthesis of the epidermal cell cycle is lowered by about 70% by Trioxsalen plus black light. From this they concluded that this combined treatment 'might have therapeutic implications for psoriasis and other skin diseases exhibiting increased DNA synthesis and rapid proliferation kinetics' (Weinstein & Frost, 1968).

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Axillary hyperhidrosis

ITS QUANTIFICATION AND SURGICAL TREATMENT

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SUMMARY

Thirteen patients were treated for their axillary hyperhidrosis by excision of axillary skin under general anaesthesia. The operation was performed by dermatologists. Significant benefit was obtained by ten of the thirteen patients. The sweat levels were measured in a control group, and in patients before operation, and at intervals up to 1 year post-operatively. This is a good operation; minor complications are common, but resolve quickly.

Excessive sweating is a socially embarrassing condition which results in psychological disturbance but rarely causes physical disease. The condition may be localized to sites such as the feet, hands or axillae, or may be generalized. In a small number of cases the hyperhidrosis is a manifestation of underlying disease, such as a neurological abnormality, an anxiety state, hyperthyroidism, phaeochromocytoma; or it may be associated with menopausal flushing. However, these conditions are usually readily diagnosed.

The majority of cases fall into the group termed idiopathic hyperhidrosis, in which the cause is unknown and the sweat glands and nerves are apparently normal. Heat, gustatory stimuli and emotional stress may result in marked increase in sweat output, as in the normal individual, or the symptom may be apparently unrelated to these factors, which stimulate physiological sweat production.

Eccrine sweat glands are innervated by cholinergic sympathetic nerves, but apocrine glands have no direct secretory innervation, though local stimulation with adrenaline will result in sweat production (Lovatt Evans, 1957).

A variety of different treatments have been used to control excessive sweating. These include anticholinergic drugs administered systemically or locally, general sedatives, radiotherapy, astringents, antiperspirant lotions containing aluminium salts, iontophoresis of various chemicals or tap water, and surgical sympathectomy. The number of different methods used is a measure of their relative ineffectiveness.

In 1962, Skoog & Thyresson noted a successful response in fifteen cases who were suffering from axillary hyperhidrosis and from whom localized areas of axillary skin were excised. Since then,

Preliminary report read at the British Association of Dermatology Annual Meeting, July 1971.

Hurley & Shelley (1963, 1966), Tipton (1968), Gillespie & Kane (1970), and Weaver & Copeman (1971) have reported success following excision of axillary skin.

The present report documents the pre-operative sweat levels in a group of patients, compared with matched control cases, and the post-operative sweat levels in these patients, 6 weeks, 4 months and 1 year after the excision of axillary skin had been performed under general anaesthesia by dermatologists.

PATIENTS AND METHODS

Thirteen patients referred to the Dermatology Department complaining of axillary hyperhidrosis, which had failed to respond to local measures or oral anticholinergic drugs, were investigated and treated. None had significant associated systemic disease and all were diagnosed as having idiopathic hyperhidrosis. Only one had troublesome sweating from other sites. The group included three men and ten women with an age range of 17–35 years (mean 25·8 years, SD ± 7·7). The controls were matched for age (mean 25·8 years, SD ± 7·7) and sex, and consisted of healthy hospital staff.

The quantification of sweating was performed in the control cases once only, but in the patients both pre-operatively, and post-operatively at 6 weeks, 4 months and 1 year. Dry cotton swabs were placed in the axillae and their weight was recorded before and after heating the individual under an electric heat cradle for 30 min. The test was quite stressful on the individual and the measurements therefore represented the response to heat and emotional stimulation. The environmental, oral and axillary temperatures were recorded during each test.

The area of maximal sweating was demarcated by the starch and iodine technique, and the skin was marked with indelible ink when the sharply circumscribed sweating area could be seen clearly. This area ordinarily measured about 8 × 4 cm. Care was taken to mark the sweating area before premedication for the operation had been given, as the anticholinergic drugs would have interfered with the result.

The operation was performed under general anaesthesia and the patients remained in hospital for 2 days post-operatively. Prophylactic oxytetracycline was administered orally and in most cases topical chlorhexidine, 1 in 2000 solution, was used to swab the wounds post-operatively to reduce infection.

An elliptical area of skin measuring approximately 2·5 × 4 cm was excised down to subcutaneous fat. The edges of the wound were rolled back and as much as possible of the subcutaneous fat and lower dermis underlying the demarcated sweating area were removed with curved scissors.

Two dermatologists operated simultaneously on the patients. The one who always performed the operation on the patient's right axilla tended to be a little more radical than his colleague and also used a vacuum drain in the wound. The drains were removed after 24 h. Care was taken to secure adequate haemostasis before the wounds were sutured. A pack of cotton wool was used to apply pressure to the axillae on the first day after operation, and the patients' arms were bandaged to their sides with arms folded whilst they were still anaesthetized at the end of the operation, and when sleeping for the night after operation.

RESULTS

Fig. 1 shows the general pattern of the results of operation on the right axilla in thirteen patients. The pre-operative sweat levels are compared with the 6 week, 4 month and 12 month post-operative levels. One case was lost to follow-up before the 12 month test. All the cases improved after operation but there was a little increase in the mean sweat levels between 4 and 12 months post-operatively.

The statistical details are shown in greater detail in Fig. 2. The very great difference ($P < 0{\cdot}01$)

between the control group (mean 1·31 g, SD ± 0·89) and the patients pre-operatively (mean 3·77 g, SD ± 2·96) contrasts with the reduction to virtually the control level at 6 weeks post-operatively (mean 1·46 g, SD ± 0·75). This reduction is highly significant ($P<0{\cdot}001$). Between 6 weeks and 4 months post-operatively, the levels did not change, but by 12 months the axillary sweat levels had increased (mean 2·28 g, SD ± 2·75), but the deterioration was not statistically significant ($P>0{\cdot}1$).

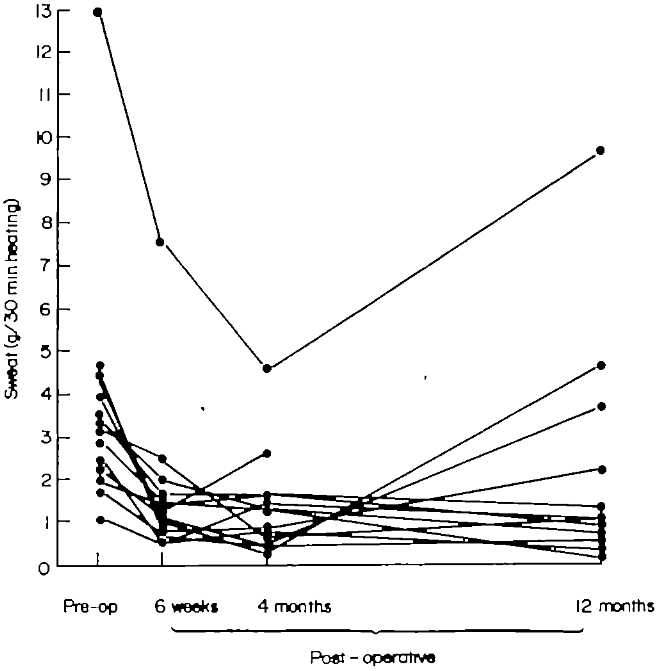

FIGURE 1. Sweat levels from the right axilla in thirteen patients pre-operatively and at 6 weeks, 4 months and 12 months post-operatively.

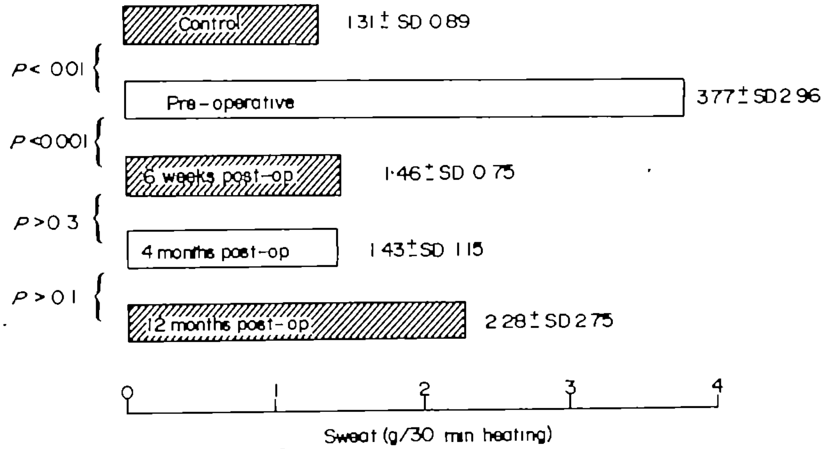

FIGURE 2. Comparison of mean sweat levels from the right axilla in thirteen control subjects and thirteen patients pre-operatively and post-operatively.

Fig. 3 shows the comparison between sweating in the right and left axillae and confirms a previous

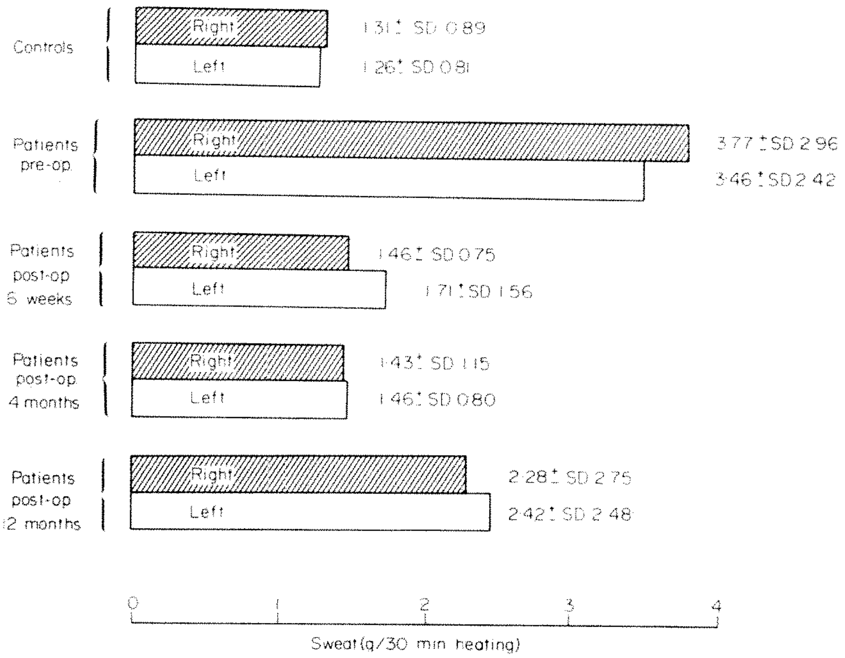

FIGURE 3. Comparison of the mean sweat levels between the right and left axilla in thirteen control subjects and thirteen patients pre-operatively and post-operatively.

TABLE 1. Complications of operation in thirteen patients

	Right	Left
Haematoma	4	1
Infection	4	2
Lymphangitis	6	2
Upper arm discomfort	3	4
Numbness	4	0
No complications	2	5

TABLE 2. Subjective assessment of operation results after 12 months in twelve patients (after 4 months in one patient)

No better	3	(23%)
Fair	3	(23%)
Good	7	(54%)

finding of slightly higher, but not significantly different, levels on the right, independent of the dominant hand of the patient. One patient and two controls were left handed, and each sweated more on the right side. The group of controls, and the group of patients pre-operatively, both showed this minimally higher sweat level on the right side. However, the post-operative measurements showed a reversal of this trend, and reflected the rather more radical operation performed by the surgeon who operated on the right axillae.

Complications of the operation were numerous, but none was serious. They were significantly higher on the right side, in which twelve patients had drains in the wounds, as compared with three on the left side. The complications are listed in Table 1; some patients had more than one. By 6 weeks after operation all complications had resolved, with the exception of mild numbness of the medial aspect of the upper arm, which settled gradually as time passed.

The patients' analysis of the results of operation at 12 months is shown in Table 2. Good results were obtained in seven (54%), fair results in three (23%), and three (23%) claimed they were no better than before operation.

DISCUSSION

The symptom from which the patients suffered is closely related to their emotional reaction and social acceptability. It is not surprising that many more women than men sought advice about the complaint. Patients with unstable or immature personalities tended to have many complaints before and after operation, a low sweat level on measurement, and a less enthusiastic assessment of their improvement after operation than those with high sweat levels, and who appeared to be of stable temperament.

Most of the patients who were classified as having good results were extremely pleased with the operation, and several no longer used topical anti-sweat preparations.

Tissue removed from the axillae at operation was examined histologically, but was found to be normal in each case. No patient complained of a compensatory hyperhidrosis of another part of the body following operation on the axilla, though this has been recorded in some patients after sympathectomy for hyperhidrosis (Shelley & Florence, 1960).

The method of quantifying the sweat output in these patients is influenced by many variables, yet seemed to give results accurate enough to be meaningful in the majority of cases. It correlated well with the patients' subjective observations.

This operation is simple and without major complication; the results are good in the majority of cases and it is a technique well within the surgical scope of dermatologists. It is considered to be too big a procedure to perform under local anaesthesia.

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Topical antibiotics in pyodermas

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SUMMARY

The value of topical antibiotics in the treatment of pyodermas was reassessed. In a double-blind study, the efficacy of 0·1% gentamicin cream was tested. There was no significant statistical difference in the degree of improvement between the group of patients that received the gentamicin cream and those treated with a placebo. The response obtained was thought to be due to washing and removal of crusts. Hence, it is considered advisable to abstain from the use of topical antibiotics in pyodermas.

Many modes of therapy have been recommended for the treatment of pyogenic infections of the skin (pyodermas). These range from simple cleaning with soap and water and removal of the crusts, to systemic administration of antibiotics. In spite of evidence that the use of systemic antibiotics is superior to topical antibiotics in the treatment of impetigo (Burnett, 1962, 1963; Huges & Wan, 1967; Dillon, 1970), topical antibiotics continue to be frequently used. The indication for this use is the superficial site of the infection, especially in impetigo where the bacteria are in the stratum corneum, the site of penetration of topically applied chemicals. However, we are aware of only one controlled study comparing the effects of topical antibiotics with those of simple washing and cleansing (Dillon, 1970). The purpose of this double-blind study is to determine the value, if any, of topical antibiotics in the treatment of pyodermas. Gentamicin cream is used as an example of such antibiotics.

MATERIALS AND METHODS

Forty-six patients included in this study were seen in the out-patient department of the American University Hospital. There were twenty-five males and twenty-one females, their ages ranging from 4 months to 65 years. Most of the subjects were children. All patients suffered from superficial pyogenic infection. Twenty-three patients had the classical clinical picture of impetigo, as described by Rook & Roberts (1972). Twenty-one of the infections were of the Tilbury Fox type and two were of the bullous type. Seventeen patients had their infection secondary to other lesions: erosions (seven cases), dermatitis (seven), insect bites (two), and perniosis (one). Six patients had superficial folliculitis. None of the patients developed any systemic manifestations, such as chills, fever or lymphadenopathy. Gram stain and cultures were done after cleaning the lesions with saline. The culture isolates were tested for their sensitivity to penicillin, pristinamycin, methicillin, cephalothin,

TABLE 1. Results after 1 week of treatment of all patients with pyoderma (including the cases of impetigo)

Agent used	No. of patients	Results		
		Excellent	Moderate	Poor
Gentamicin	21	11 (52%)	5 (24%)	5 (24%)
Placebo	25	12 (48%)	7 (28%)	6 (24%)
Total	46			

TABLE 2. Results after 1 week of treatment of patients with classical impetigo

Agent used	No. of patients	Results		
		Excellent	Moderate	Poor
Gentamicin	11	6 (55%)	2 (18%)	3 (27%)
Placebo	12	6 (50%)	3 (25%)	3 (25%)
Total	23			

TABLE 3. Organisms cultured from skin lesions prior to treatment

Organism	Gentamicin group	Placebo group	Total
Staphylococcus aureus			
Coagulase positive	17	18	35
Coagulase negative (DNAse pos., mannitol pos.)	0	1	1
Staphylococcus albus	0	1	1
Beta-haemolytic streptococcus (group A)	10	9	19
Alpha-haemolytic streptococcus	0	1	1

vancomycin, gentamicin, and tetracycline by the Kirby–Bauer disc agar diffusion technique (1966). Coded tubes containing 0·1% gentamicin cream or placebo (vehicle without gentamicin) were provided by the manufacturer. The medications were given at random and the patients were instructed to wash the lesions with a 2% hexachlorophane soap twice daily and to remove crusts when these were present. The cream was then applied liberally over the affected areas twice daily. In the two patients who had bullous impetigo, the bullae were ruptured during the first visit. All patients were seen at weekly intervals and were instructed not to wash the lesions or apply the cream for at least 12 h before coming for follow-up. Those who did not have complete clearance of their lesions at the end of the first week of treatment were given systemic antibiotics and/or topical steroids when there was an underlying dermatitis. Therefore, the evaluation of improvement had to be limited to the first week of therapy. The results were assessed as follows: (1) excellent (complete disappearance of the lesions); (2) moderate (improved but not completely cleared); (3) poor (minimal or no improvement).

RESULTS

The results after 1 week of treatment are summarized in Tables 1 and 2. There is no significant statistical difference between the group of patients with pyoderma who were treated with the gentamicin cream and those who received the placebo cream ($P > 0{\cdot}05$). Similarly, there is no statistical difference in the two treatment schedules in the sub-group of patients who had classical impetigo lesions. Follow-up on the cases who had the excellent response did not reveal any recurrence of the lesions up to 3 weeks after the last day of treatment. The appearance of lesions elsewhere on the body was observed in three patients treated with the gentamicin cream and in two patients who received the placebo cream. No untoward side effects were observed with the gentamicin cream.

The organisms isolated from the lesions, and their relative frequency are listed in Table 3. All organisms cultured from the lesions were sensitive to gentamicin. 89% of *Staphylococcus aureus* strains were resistant to penicillin, while 43% were resistant to tetracycline. 21% of the group A beta-haemolytic streptococci were resistant to tetracycline. The placebo cream was found to have no antimicrobial effects by *in vitro* tests.

DISCUSSION

Our main concern in this study was to determine whether topical antibiotics play an important role in the treatment of superficial bacterial infection of the skin. Gentamicin was chosen as an example because it is known to be an effective antibiotic (Taplin, 1972), and clinical studies have proved it to be at least as effective topically as neomycin (Farah *et al.*, 1967). Davis, Fulgum & Taplin (1968) and Lloyd (1969) found that the addition of neomycin to corticosteroid preparations did not enhance the effect of the steroids used topically on infected and inflammatory eczematous lesions. Our results show that there is no statistical difference between the response of patients treated with gentamicin cream and those treated with a placebo, though all the organisms recovered from the treated skin lesions were sensitive to gentamicin. Gentamicin cream did not prevent the appearance of new lesions in other sites while the patients were still under treatment.

These findings indicate the ineffectiveness of topically applied antibiotics in the treatment of pyodermas. If, in addition, one considers the risks of sensitization and of the possible emergence of resistant organisms, it seems doubtful whether topically applied antibiotics serve any useful purpose and it is evident that they could be potentially harmful.

A high percentage of the *Staph. aureus* strains was resistant to penicillin. Similar results were reported by Dajani, Farah & Kurban (1968), who found 85% resistant to penicillin. 21% of the beta-haemolytic streptococci were resistant to tetracycline, and this is in agreement with previous reports (Brorson, Roupe & Seeberg, 1972).

In conclusion, the clinical usefulness of topical antibiotics, exemplified by gentamicin, in the treatment of superficial infections of the skin is doubted. The response obtained is most probably due to washing, and removal of the crusts. It is, therefore, advisable to abstain from the use of topical antibiotics in pyodermas.

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Cryoprobe treatment of acne conglobata

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SUMMARY

Twenty-five patients with acne conglobata were treated with cryotherapy delivered via probes. The probe was applied to nodular lesions for 20–25 s without pressure. Freon, carbon dioxide and nitrous oxide were found to be therapeutically equivalent. Nodulo-cystic lesions resolved within 7–10 days leaving a thin atrophic scar on the back and no scar on the face. The treatment was almost painless; no recurrence has appeared in the treated areas.

The carbon dioxide slush technique provided a form of cryotherapy which was helpful in acne vulgaris (Karp, Nieman & Lerner, 1931). This procedure was troublesome, however, and it is difficult to control the exposure. Recently, Graham has used liquid nitrogen spray to advantage. In her hands, spraying every 2–4 weeks produces enough desquamation to be useful in treating all varieties of acne lesions and even in post-acne scarring (Graham, 1971). Refrigerants, however, may also be delivered via cryoprobes in which the refrigerant does not contact the skin. This form of cryosurgery has become popular for treatment of benign cervical lesions (Ostergard, Townsend & Herose, 1969), tonsillectomy (Rabkin, 1968), and retinal detachment (Shea, 1967).

It is the intent of this report to demonstrate the usefulness of cryoprobe therapy as an adjunct in the treatment of acne conglobata, the severest type of adolescent acne. We had only modest success with the cryoprobe in eliminating comedones and in clearing papulo-pustular acne. The temperature decrease with the cryoprobe is not great enough to bring about a sufficient degree of peeling to influence the common or garden varieties of acne vulgaris. By contrast, cryoprobe therapy was surprisingly useful for the large, indolent, nodular lesions of acne conglobata. Acne conglobata often responds poorly to antibiotics and traditional peeling agents. Local injection with steroid solutions is unquestionably beneficial, but may be trying for the doctor and patient when there are many lesions. In addition, adrenal suppression becomes a consideration when large amounts are injected (Potter, 1971).

MATERIALS AND METHODS

Twenty-five patients from the Acne Clinic of the Hospital of the University of Pennsylvania were treated solely with cryoprobe therapy. All had severe nodulo-cystic acne. Three refrigerants were used and found to be therapeutically equivalent: carbon dioxide, nitrous oxide and freon. These

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agents achieve temperatures in the −60 to −80° C range. There are several cryo-units available which deliver these refrigerants via probes, with tips of various shapes and sizes. Probes with a flat-surfaced tip with a diameter of about 10 mm proved to be the most satisfactory. From an engineering standpoint the carbon dioxide unit was easiest to work with, particularly in that the tank did not need as frequent changing as it did in the other units.

The technique consisted of placing the probe on the lesion without pressure and freezing for 20 s. For lesions larger than 1 cm, the probe was successively applied until the entire lesion was frozen. Patients were followed weekly, and new or incompletely resolved lesions were treated.

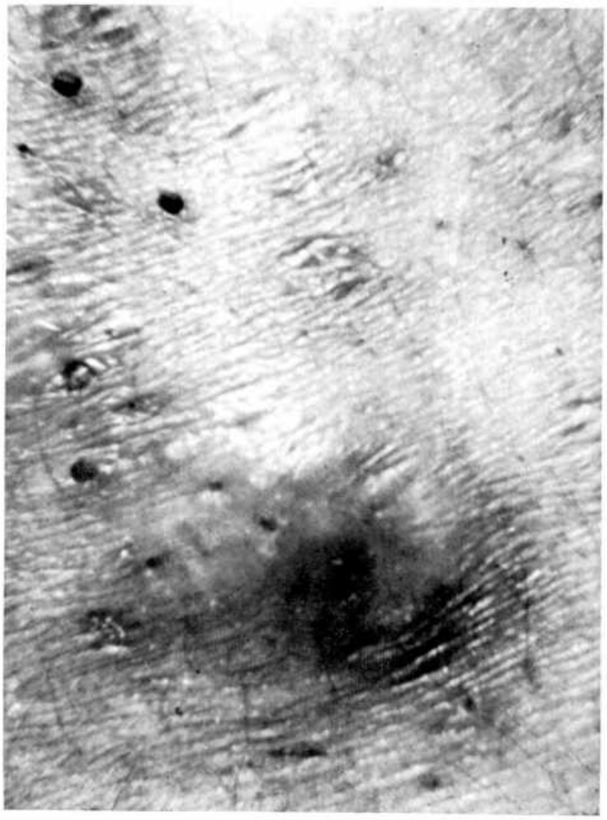

FIGURE 1. Indolent, nodular acne lesions before cryotherapy.

RESULTS

A single application of the probe usually resulted in marked flattening or complete resolution of nodulo-cystic lesions in 7–10 days (Figs. 1 and 2); spontaneous regression of untreated lesions rarely occurs in less than a month. During freezing the lesions blanched; immediately after there was often a transient urticarial reaction. Slight crusting developed during the first 3–4 days and by 7–10 days the nodule was replaced by a flat, slightly atrophic scar. Occasionally, an unusually firm nodular lesion required a second freezing. There has been no recurrence in treated areas during a follow-up period of 12–28 months. Facial lesions treated in this fashion leave barely perceptible scars. The scars on the chest and back are less acceptable cosmetically but similar to those which eventuate without treatment. We would emphasize that this procedure is practically painless and is very acceptable to patients.

Temperature changes during cryotherapy

Tissue temperatures were monitored with thermocouples during therapy of eight patients. The thermocouple needles were placed at approximately 2 mm beneath the surface and just below the lower pole of the nodule. The lesions were frozen for 25 s. The temperature was observed during application and after removing the cryoprobe. The results are summarized in Table 1. The average temperature drop at the 2 mm level was 23° C during the 25 s freezing period. The temperature decrease just below the lesions was only about 8° C. 2 min following freezing, the temperature was only slightly lower than the baseline reading.

TABLE 1. Temperature recordings during cryotherapy of cystic acne

		Temperature (°C)			
			Time		
Case no.	Depth of thermocouple needle (mm)	Baseline temperature	15 s	25 s	2 min past freezing
1	2	35	12	6	20
	7	35	26	18	26
2	2	34	0	−10	+10
	8	35	25	20	26
3	2	34	0	−5	26
	7	35	28	21	30
4	2	33	10	−6	20
	6	36	30	20	28
5	2	33	0	–	29
		35	6	–	22
6	2	33	20	10	–
	7	34	24	18	–
7	2	32	8	0	20
	8	34	24	12	30
8	2	34	11	–	–
	10	35	29	–	–

Histopathology

Six patients were biopsied at various intervals following cryotherapy. Biopsies of untreated lesions showed a deep dermal abscess composed of a dense mass of granulocytes and some mononuclears. Foreign body giant cells were usually present. Some specimens revealed a ruptured comedo with surrounding far flung inflammation, whilst in others only remnants of odd-shaped follicular epithelium were found (Fig. 3).

Twenty-four hours after 25 s of freezing, the epidermis showed varying degrees of necrosis, especially in the upper portion. Many cells had hyperchromatic, pyknotic nuclei. Intracellular and intercellular oedema was evident. The dermis appeared oedematous and seemed, therefore, to bring about dispersion of the inflammatory infiltrate.

By 48 h there was complete sloughing of the epidermis with crust formation. Collagen bundles were separated by oedema and granulocytes seemed not so tightly packed, though there was no sign of their having been killed outright (Fig. 4).

At 72 h the crust covered an acanthotic epidermis which clearly showed signs of regeneration with large basophilic cells with prominent nuclei. The dermis again showed mainly oedema and dispersion of the inflammatory infiltrate.

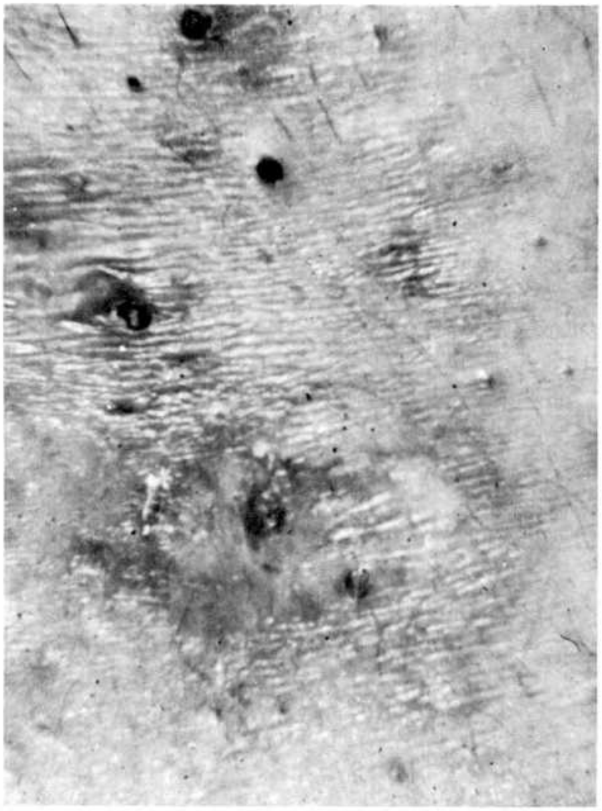

FIGURE 2. One week after a 15 s period of freezing with CO_2 delivered via a cryoprobe.

By 7–10 days the epidermis appeared normal. The dermo-epidermal junction was flat, with obliteration of the papillary body. In the dermis, there was total loss of neutrophils and only occasional mononuclear cells were seen. Many stellate fibroblasts were found in a stroma of thin bundles of collagen and numerous dilated venules (Fig. 5). Elastic fibres were entirely obliterated at the site of the former abscess (Orscein stained).

DISCUSSION

Refrigerants delivered via probes offer an effective therapeutic manoeuvre in the treatment of stubborn nodulo-cystic lesions. The mode of action is not entirely clear. The lowered temperatures achieved do not begin to approach the −20° C range which has been shown to be necessary to irradicate malignant skin tumours (Taylor, 1957; Cooper, 1965). Thermocouple readings showed an average temperature drop of 23° C at a 2 mm level and at only 8° C just below acne nodules. The resolution of nodulo-cystic lesions was evidently not a result of direct killing of inflammatory cells. Necrosis occurred only in the upper part of the epidermis and not below this level. One might speculate that even modest lowering of the temperature could damage lysosomal membranes sufficiently to liberate hydrolytic enzymes. These could then liquefy tissue constitutents and enhance removal of inflammatory products. Cunliffe demonstrated inhibition of fibrinolytic activity in inflamed skin (Cunliffe *et al.*, 1971): this could lead to fibrin deposition and interfere with movement of cells and materials. Perhaps cryotherapy stimulates fibrinolysis in some unknown manner. Another factor is reactive vasodilation resulting in an increased blood flow; perhaps the most likely explanation. The latter would certainly promote clearance of toxic products. We tested this idea provisionally by injecting 0·5 ml of a 5% aqueous solution of sodium fluorescein into normal skin and into two nodular lesions

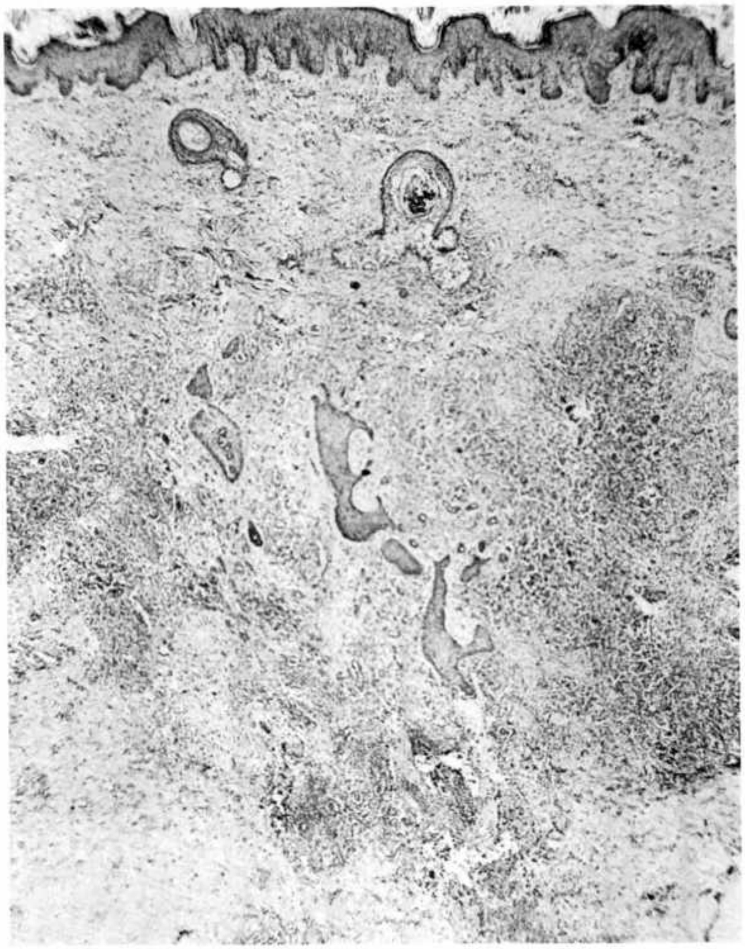

FIGURE 3. Acne nodule showing a deep abscess in the dermis with follicular remnants surrounded by a dense infiltrate of polymorphonuclear leukocytes along with mononuclear cells and giant cells.

in five patients. Under Wood's light the injected nodules fluoresced brilliantly. The cryoprobe was applied to one nodule for 20 s and the normal skin went untreated. In normal skin no fluorescence could be detected after 3–4 days. Untreated nodules still fluoresced at 12 days. Cryotherapy reduced the clearance time to 6 or 9 days. An increased blood flow through the area would be the simplest way to explain the more rapid clearance of this freely diffusable dye. Doubtless there are chemical as well as mechanical aspects to this effect.

Steroid injection was compared with cryotherapy in three patients with nodular lesions. A total of 0·5 ml of 10 mg/ml triamcinolone acetonide solution was injected into one nodule while a comparable lesion was frozen for 20 s. Resolution of cystic lesions occurred in 3–4 days after steroid injection compared to 7–10 days after cryotherapy. Thus injection of steroids is swifter in its action. Despite this, the painless nature of cryoprobe therapy makes it attractive, especially when there are many lesions. A disadvantage of steroid injection is that recurrence within a few weeks is not an uncommon phenomenon. Cryotherapy produces a thin atrophic scar but not the extensive atrophy which steroid injections occasionally produce.

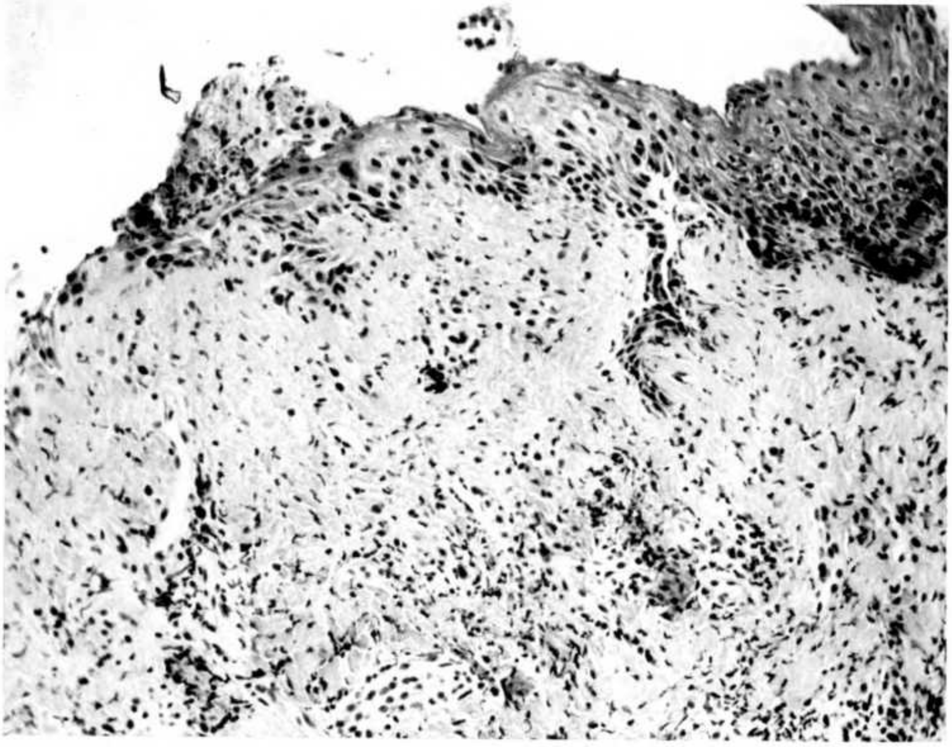

FIGURE 4. Acne nodule 48 h after a 25 s application of CO_2 via cryoprobe. The epidermis has partially sloughed with other areas demonstrating intercellular and intracellular oedema. In the dermis there is a marked dispersion of the normally dense infiltrate along with oedema of the collagen.

Finally, as regards Graham's advocacy of liquid nitrogen sprays to treat acne vulgaris, we shall merely summarize our own limited experience. Graham's claims are veritable; repeated spraying can indeed produce sufficient peeling to bring ordinary forms of acne under reasonably good control. Pain and discomfort during spraying are serious objections, however, and we encountered a great deal of patient resistance and defection from scheduled appointments.

Currently, we use refrigerants only to treat indolent nodular lesions by the technique described.

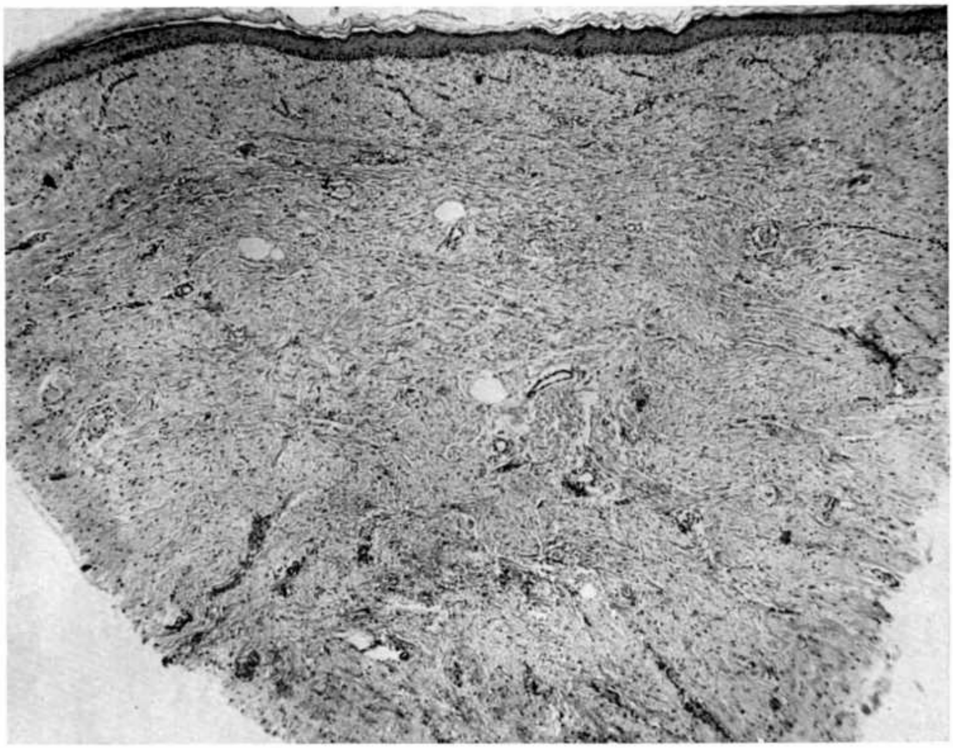

FIGURE 5. One week after a 25 s freezing of an acne nodule. A proliferation of stellate fibroblasts has replaced the acne abscess.

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Case Reports

Intra-epidermal epithelioma

REPORT OF CASE WITH INVASION AFTER MANY YEARS

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SUMMARY

An intra-epidermal epithelioma of 30 years duration invaded as squamous cell carcinoma. The tumour exhibited an unusual propensity for the production of parakeratotic debris. The potential of intra-epidermal epithelioma for invasion is reviewed.

It no longer seems useful to attribute any diagnostic or prognostic specificity to the designation 'Borst–Jadassohn epithelioma' (Mehregan & Pinkus, 1964; Holubar & Wolff, 1969; Holubar, 1969). Rather, Pinkus & Mehregan (1964) have proposed that three different phenomena with intra-epidermal nests be designated by the names Borst, Jadassohn and Paget respectively. The Borst phenomenon is nest formation by intra-epidermal invasion from an adjacent squamous cell carcinoma or Bowen's disease. The Jadassohn phenomenon is the intra-epidermal origin of distinct nests of keratinocytes which long remain confined to the epidermis. The Paget phenomenon is the invasion of the epidermis, with nest formation, by non-keratinocytic tumour cells; instances include mammary and extramammary Paget's disease, malignant melanoma and epidermotropic metastatic adenocarcinoma of the bladder. Although this Paget phenomenon is of great biological interest, and often a concern in differential diagnosis, it does not require further consideration in this paper. This case report documents the development of invasive squamous cell carcinoma in a recurrent 'intra-epidermal epithelioma' which initially exhibited only the Jadassohn phenomenon. The formation of unusual masses of parakeratotic debris is also emphasized.

CASE REPORT

In 1959, a 69-year-old white woman was hospitalized with multiple injuries following a road accident. She drew attention to an asymptomatic lesion which had been present on the left calf for 20 years. There was no history of arsenic ingestion. The 5 × 5 cm plaque was generally raised and firm, focally scaly and centrally ulcerated. The initial clinical impression was superficial basal cell epithelioma. A punch biopsy was reported as 'intra-epidermal epithelioma of Borst–Jadassohn'. The lesion was destroyed by curettage and fulguration.

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Ten years later (1969) this patient returned stating that in 1961, 2 years after the initial treatment, she had noticed a recurrence of the 'warty growth' in the same area of the left leg. Within the past 2 months, she had observed the rapid growth of a vascular nodule within this scaly area. Examination now revealed a large, crescent-shaped, hyperkeratotic plaque, 10 cm in diameter and with a discrete, circular, vascular nodule 3 cm in diameter at the lower pole (Fig. 1).

General examination was within normal limits for her age, without adenopathy or stigmata of arsenic ingestion.

The entire mass was excised to the deep fascia, together with a 2 cm margin of normal skin. The defect was covered with a split thickness graft. No local recurrence or distant spread had occurred by the time of her death from other causes in 1973.

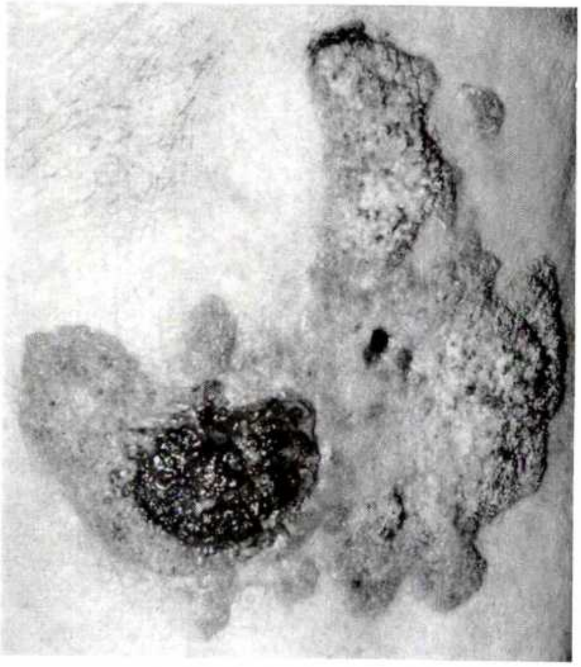

FIGURE 1. 1969. Recurrent 'intra-epidermal epithelioma' 10 × 10 cm with central squamous cell carcinoma.

Histopathology

The 1959 biopsy shows irregular hyperkeratosis and focal parakeratosis on the surface and, within the well defined epidermal nests, discrete parakeratotic masses. The elongated rete ridges, without atypical features, branch and anastomose freely. Some of the nests of atypical cells are more eosinophilic and 'squamoid', while others are more basophilic than the surrounding epidermis. Fig. 2 demonstrates a well defined 'squamoid' nest. Some of the atypical cells appear to be forming pearls, while others are undergoing individual cell keratinization with the early formation of parakeratotic debris. Some whorls resemble 'squamous eddies'. In Fig. 3 (a high power view of the nest in Fig. 2) are several adjacent cells, with condensed eosinophilic cytoplasm and pyknotic nuclei resembling grains, which produce the parakeratotic debris, without transition through a normal granular layer. Fig. 4 shows low in the epidermis a mass of this debris, which in other sections is extruded into the stratum corneum.

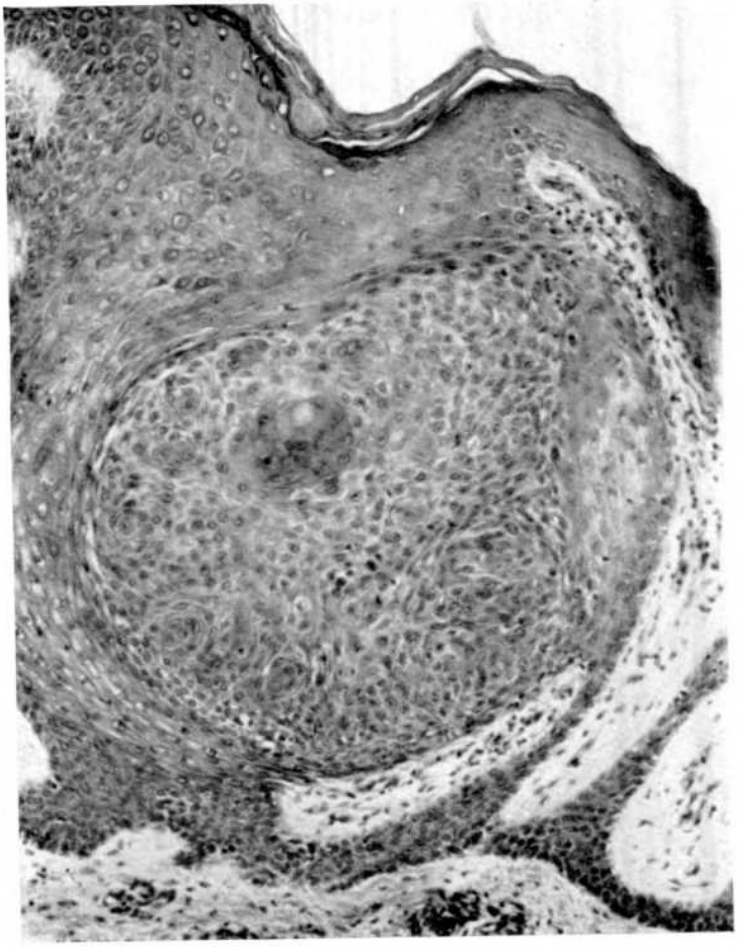

FIGURE 2. 1959. A 'squamoid' nest of atypical cells.

In the peripheral material excised in 1969, areas similar in all respects to those in the 1959 biopsy are noted. However, the central friable raised mass seen grossly in Fig. 1 is invasive squamous cell carcinoma. The superficial portion of this area has more cellular variation and less tendency toward nest formation than have sections from the periphery; deep to this area the sheets of atypical squamous cells extend to the level of the eccrine sweat glands, where they still retain the ability to produce the masses of parakeratotic debris. In fact, the sheets and masses of parakeratotic debris are so extensive that in some areas the nests of squamous cells appear as islands in a sea of debris (Fig. 5).

PAS stain revealed insignificant quantities of glycogen both in the better differentiated nests and in the poorly differentiated sheets of tumour cells. There was no accumulation of acid mucopolysaccharide demonstrable by the Alcian blue or colloidal iron techniques.

DISCUSSION

The type of nesting of keratinocytes in the 1959 biopsies and at the periphery of the 1969 material

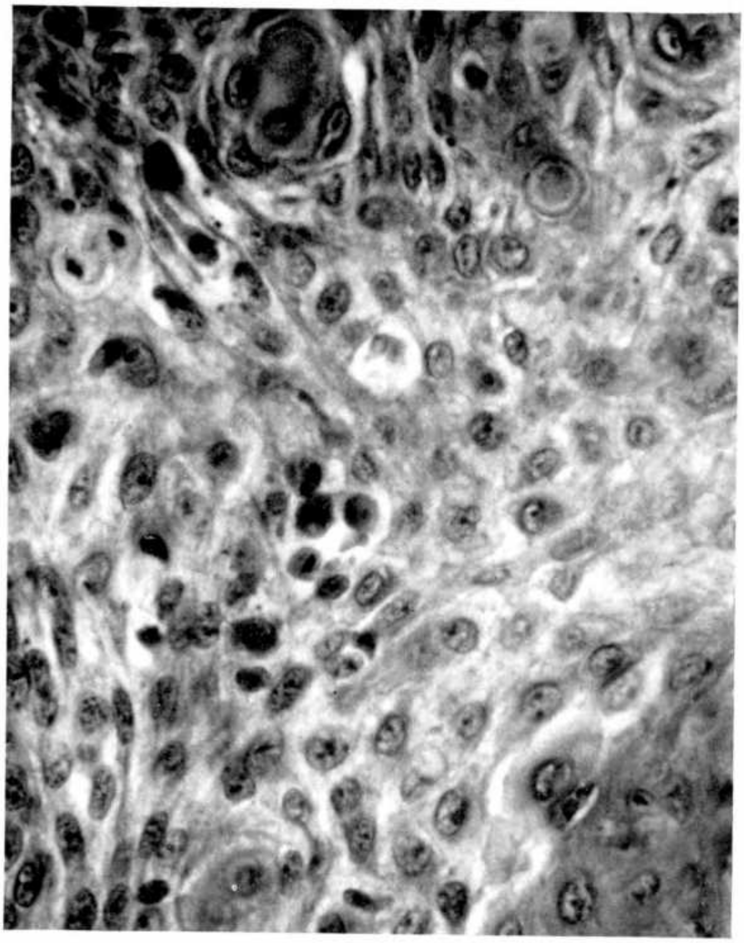

FIGURE 3. 1959. Central 'grains' which form parakeratotic debris.

demonstrates the Jadassohn phenomenon. Except in seborrhoeic keratoses or epidermal naevi, in which the nests are less sharply defined, most examples of the Jadassohn phenomenon represent hidroacanthoma simplex (Smith & Coburn, 1956) derived from the acrosyringium. The tumour reported here, however, is unlikely to be related to hidroacanthoma simplex but is more probably derived from epidermal keratinocytes. In hidroacanthoma simplex there is usually abundant glycogen (Holubar & Wolff, 1969), demonstrable by PAS stain, but our material produced insignificant quantities. Even malignant hidroacanthoma simplex (Ishikawa, 1971) or porocarcinoma (Mishima & Morioka, 1969) retain the ability to produce glycogen and clefts or ductal structures. Furthermore, keratinization as demonstrated in Fig. 5 is not seen in malignant tumours of the acrosyringium.

Our case probably represents a variant of Bowen's disease. The character of the dyskeratotic change, initially confined to the nests, and later seen in the invasive process, resembles most closely that type of carcinoma-*in situ*. Haber's first case (1958) was a tumour histologically similar to this case. He emphasized that the nests seem to be 'expelled by the normal course of keratinization, breaking through the granular layer and appearing within the horny layer in the form of parakeratotic cells.' The tumour he reported apparently also invaded pilosebaceous units, a characteristic of Bowenoid

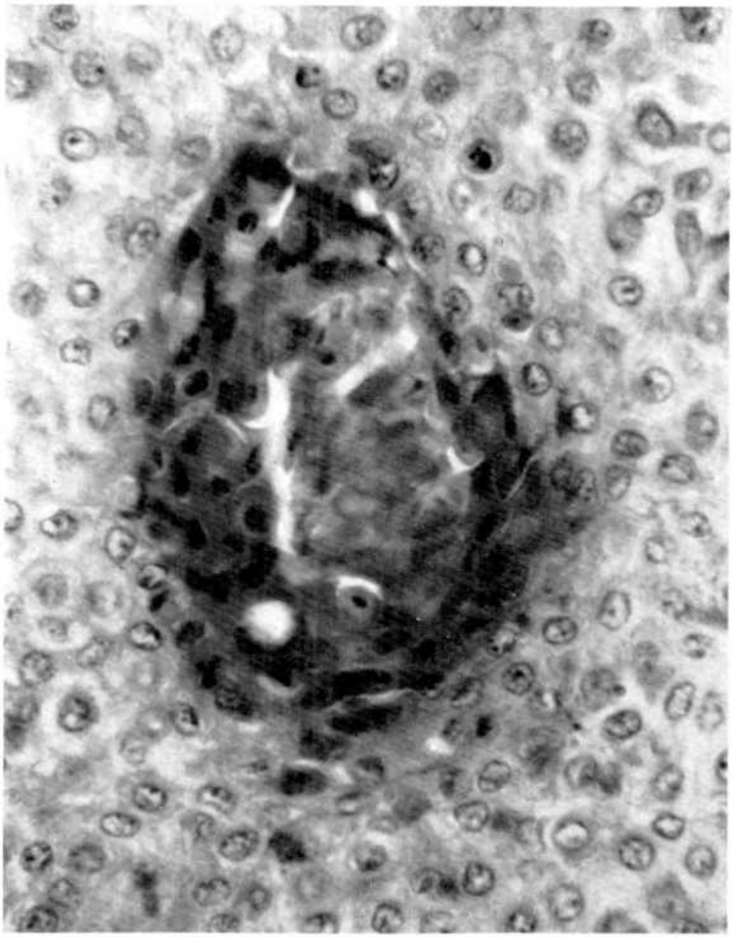

FIGURE 4. 1959. Parakeratotic mass within nest in lower epidermis.

carcinoma-*in situ*. Therefore, although the initial tumour (1959) was not considered an example of the Borst phenomenon, i.e. neither squamous cell carcinoma nor Bowen's disease could be diagnosed at that time, the histology of its subsequent invasion is most consistent with that of Bowen's disease when it invades as squamous cell carcinoma.

Ordinarily, tumours with the Jadassohn phenomenon are considered to have little invasive potential. The published reports of invasive carcinoma from 'intra-epidermal epithelioma' probably represent more than one type of tumour. Tappeiner & Pfleger (1964) diagnosed intra-epidermal epitheloma in four of 1500 specimens in 5 years. Two of these were considered malignant: one with parakeratotoic debris similar to this case, the other with a more glandular differentiation. Graham & Helwig (1959) reported six instances of invasion by 'intra-epidermal epithelioma of Jadassohn' in material from forty-three men and thirty-three women, with one death resulting. They mentioned, in some, spotted parakeratosis and horn cysts as well as the fact that intra-epidermal nests could be basaloid or squamoid.

The eponyms Borst and Jadassohn are still useful individually applied to the phenomena delineated by Mehregan & Pinkus (1964), but not when combined to describe a tumour. The term

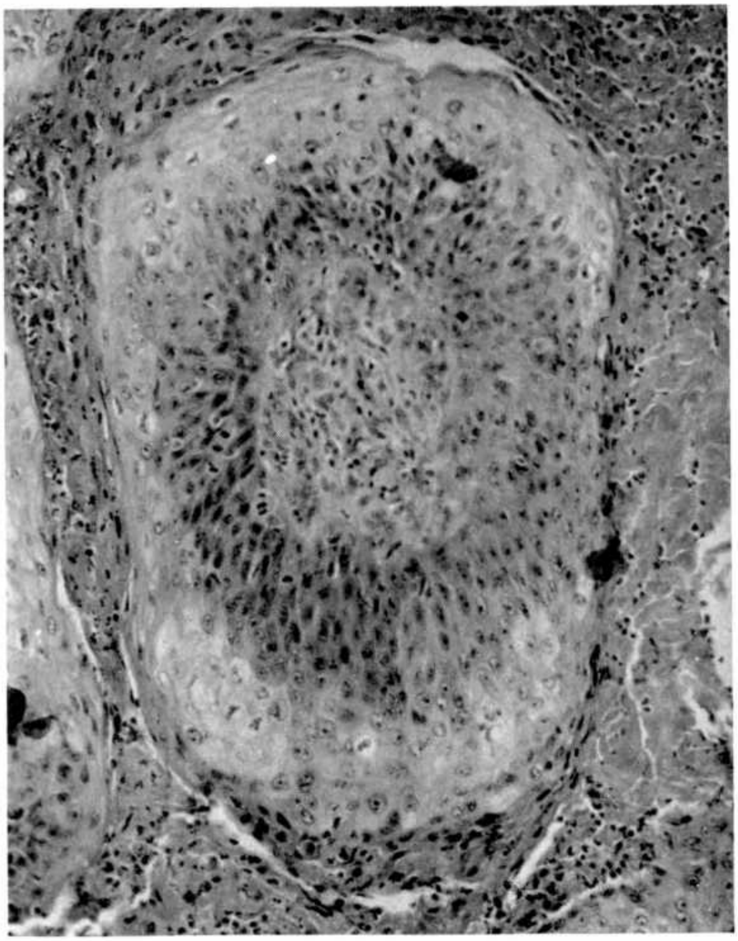

FIGURE 5. 1969. Nest of atypical squamous cells as 'island in sea of parakeratotic debris'.

'intra-epidermal epithelioma' is still valid when applied to the type of tumour reported here; this began with intra-epidermal nests of atypical cells and displayed its potential for invasion as squamous cell carcinoma after many years.

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Cutaneous leiomyomata in two brothers

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SUMMARY

A patient with multiple cutaneous leiomyomata of pilar origin is described. The hereditary aspects of the condition are stressed and the clinical features and histopathology are briefly reviewed.

Cutaneous leiomyoma is an uncommon tumour arising either from arrector pilorum muscle in the dermis or from smooth muscle of mammary glands, scrotum, labia majora or the wall of blood vessels. There are many reports of patients with leiomyomata in the literature, but in only a few instances has the true familial incidence of this disease been demonstrated. Kloepfer *et al.* (1958) and Fischer & Helwig (1963) suggested that the disorder is familial. Recently, we have come across a family in which two brothers had cutaneous leiomyomas, suggesting that the disorder may have an hereditofamilial origin.

CASE REPORT

S.C., a 26-year-old man, visited our out-patient department in July 1972 complaining of multiple nodular lesions scattered over the body for the previous 6 years. The lesions were gradually increasing in number and size and had not responded to any treatment prescribed. The lesions became painful on sudden changes in the environmental temperature. There was no complaint suggesting systemic involvement.

In his family, one of his three brothers was found to have a similar disorder. He also had had painful nodular lesions on the arms and trunk for the previous 10–12 years. The nodules, though fewer in number, were exactly the same in appearance. The patient also had one healthy sister. His parents were free from any disease and there were no other affected members in the family.

General physical examination revealed no abnormalities. In the skin were multiple firm nodular lesions affecting the right arm, the scapular regions, the lower back, the right hip and the front of the chest. The nodules were mobile, skin coloured and were mostly distributed in groups. The size of individual nodules varied from 5 mm to 1 cm. The nodules were tender to slight pressure.

Investigations

Blood picture, X-ray of chest and of right arm and hand, urine analysis: all normal.

Histopathology (Fig. 1). The epidermis was thinned over the lesion from which it was separated by a continuous layer of normal dermis. The lesion consisted of interlacing bundles of elongated cells with elongated nuclei with prominent proliferation. The tissue was uncapsulated. A Van Gieson stain showed yellow coloured bundles suggesting muscle tissue. With the Mallory stain the tumour tissue stained red and the connective tissue stroma stained blue. Thus the diagnosis of cutaneous leiomyoma was confirmed.

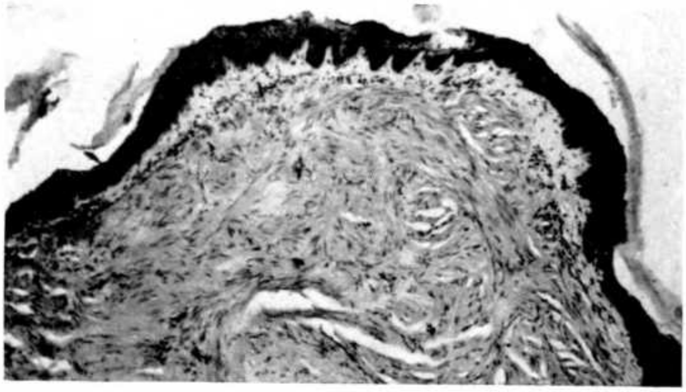

FIGURE 1. Shows thinned epidermis. The tumour is separated from the epidermis by a layer of normal dermis. The tumour consists of interlacing bundles of elongated cells with elongated nuclei (H & E, × 70).

DISCUSSION

Cutaneous leiomyoma appears clinically in the following forms: multiple cutaneous leiomyoma of pilar origin; multiple cutaneous leiomyoma of mammary origin; solitary cutaneous leiomyoma of pilar origin; solitary genital leiomyoma; solitary angioleiomyoma.

Patients with multiple lesions outnumber patients with solitary lesions in a ratio of 3:1. If the mammary leiomyomata are excluded the ratio becomes 7:1 (Fischer & Helwig, 1963). The lesions arise as multiple, firm dermal papules and nodules which are pink to red in colour and vary in size from 5 mm to 23 mm. They are fixed in the skin but freely mobile on the underlying structures. They are often, but not always, tender (Montgomery & Winkelmann, 1959). The tumours are usually bilateral and are distributed singly or in groups. In our patient the lesions were multiple and most were in groups. The disorder seems to be hereditofamilial. Kloepfer *et al.* (1958) were the first to report certain families in which more than one person was affected. Later Fischer & Helwig (1963) described the familial incidence of this disorder in some of their patients. Rudner, Schwartz & Grehin (1964) reported this tumour in identical twins. Kloepfer *et al.* were of the opinion that inheritance of this disease is determined by an incompletely penetrant autosomal dominant gene. The present report of the disease in two brothers in a single family also suggests the hereditary origin of this disorder, though it throws no further light on the mode of inheritance.

The course is benign but progressive. The recurrence rate after excision is more than 50% (Fischer & Helwig, 1963). Older lesions grow in size and finally become stable. Spontaneous resolution has not been reported.

The histology of the condition is characteristic (Lever *et al.*, 1967). The epidermis is either normal or slightly atrophic. There is a narrow clear subepidermal zone above the tumour which is usually in the mid-dermis. It is uncapsulated and is seen either to infiltrate the deeper structures and skin adnexae or to push them aside. The tumour consists of bundles of muscle tissue and collagen fibres. There is irregularity of size and arrangement of cells in the lesion. The nuclei are thin, long and rod-shaped. The muscle cells are fibrillated and vacuolated and fissures are seen in the stroma. All these features were clearly evident in our case.

ACKNOWLEDGMENTS

We are grateful to Dr Prem Chandra, Director Principal, Medical College and Hospital, Rohtak, for allowing us to publish this report.

Our thanks are also due to Dr Ramesh Arya, Assistant Professor of Pathology, for histopathological work and photomicrography.

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Comment

NEW CONCEPTS IN THE MANAGEMENT OF MYCOSIS FUNGOIDES

Mycosis fungoides is a rare form of malignant lymphoma that originates in multiple foci in the skin but eventually progresses to involve multiple extracutaneous sites, such as lymph nodes, spleen, bone marrow and other organs. Since its description in the beginning of the nineteenth century, it has been considered as invariably fatal; patients usually succumb either to severe skin infections and septicaemia, or to generalized, extracutaneous lymphomatous disease.

Over the past 20 years, two methods of treatment have been shown to be extremely effective in providing control of skin lesions of mycosis fungoides: total skin irradiation with 2·5 MeV electrons (Fuks, Bagshaw & Farber, 1973) and whole body topical application of dilute solutions of nitrogen mustard (Van Scott & Kalmanson, 1973). In a recent report of the experience at Stanford (Fuks *et al.*, 1973), it was shown that 58% of 132 patients treated with 2·5 MeV electrons attained a complete remission of their cutaneous manifestations. Fourteen patients gained freedom from disease for periods varying from 3 to 14 years, following a single course of treatment, and to date none of these has relapsed. It seems reasonable to believe that these patients initially had disease which was limited to the skin and has been cured by the treatment.

Long term remissions, of 2–4 years, have also been reported to be feasible by whole body topical application of nitrogen mustard (Van Scott & Kalmanson, 1973). However, in order to sustain remission, permanent maintenance therapy was required, and this was further complicated by the fact that a significant fraction of the patients developed hypersensitivity reactions to the drug. The curative potential of topical application of nitrogen mustard is yet to be established, and at present only electron beam irradiation can be considered curative of mycosis fungoides limited to the skin. It seems justified to state that all patients in whom mycosis fungoides is confined to the skin, should be treated as early as possible with electron beam irradiation and with the expectation of cure rather than of more temporary symptomatic relief.

Extracutaneous mycosis fungoides connotes a grim prognosis. Treatment programs for extracutaneous disease have been, in most cases, disappointing, and the majority of patients die within 1 year following the diagnosis of extracutaneous extension. However, in many mycosis fungoides patients it seemed possible to diagnose a premalignant state in the lymph nodes. More than half of the patients have on initial physical examination lymphadenopathy which on histological examination most often reveals benign changes of 'dermatopathic lymphadenitis' (Fuks *et al.*, 1973). A statistically significant difference in survival was observed by actuarial analysis between patients with generalized plaque disease who had significant lymphadenopathy prior to irradiation and those who did not. Survival of those patients presenting with no lymphadenopathy, at risk for 5–10 years following treatment, was 67·6%, and for those with initially enlarged lymph nodes was 43%. Only one of twenty-six patients with no initial lymphadenopathy eventually developed extracutaneous dissemination, compared to ten of twenty-eight who initially had enlarged lymph nodes. It seems that in mycosis fungoides, dermatopathic lymphadenitis and nodal involvement with disease are circumstantially, if not aetiologically, associated and that dermatopathic lymphadenitis may indicate presumptive nodal involvement with disease. If improved prognosis of advanced mycosis fungoides is to be pursued, early diagnosis of premalignant and malignant extracutaneous involvement as well as the establishment of new philosophies of treatment will become absolute necessities.

On the basis of these observations and assumptions a new staging classification for mycosis fungoides has been suggested (Fuks *et al.*, 1973) as follows:

Stage 1. Mycosis fungoides confined to the skin only.
Stage 2. Mycosis fungoides confined to the skin, with dermatopathic changes in regional lymph nodes.
Stage 3. Mycosis fungoides confined to the skin and regional lymph nodes and/or the spleen.
Stage 4. Cutaneous and extracutaneous mycosis fungoides with or without lymph node and/or splenic involvement.

This classification requires assessment of the full extent of involvement, which can be done by carrying out a series of detailed clinical, radiological, surgical (including staging laparotomies) and histopathological diagnostic procedures, such as is now utilized for the staging of Hodgkin's disease and other lymphomas. Such procedures have not yet been used extensively in mycosis fungoides patients. The proposed staging classification could provide some rational basis for using new treatment approaches such as total nodal irradiation for presumptive or established nodal disease, and various combinations of radiotherapeutic and multiple drug chemotherapeutic programs for established extracutaneous involvement. It is hoped that the introduction of such new treatment techniques, combined with the already available effective methods for controlling cutaneous manifestations, will improve the grim prognosis of advanced mycosis fungoides.

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British Journal of Dermatology (1974) **90,** 358.

Obituary

THOMAS EDGE ANDERSON

Dr T.E. Anderson, formerly Senior Dermatologist to the North Eastern Hospital Board, Scotland, and Senior Lecturer in Dermatology to the University of Aberdeen, died on 21 September 1973.

Dr Tom Anderson practised dermatology in the North East of Scotland for the best part of 40 years. He was born in 1903 in Odessa, where his father was Superintendent of the Eastern Telegraph Company. He completed his schooling in Aberdeen Grammar School and continued to Aberdeen University, graduating M.B.Ch.B. with honours in 1926. He obtained the Murray Gold Medal as most distinguished student of the year. In 1931 he completed his M.D. with honours, and subsequently became a Fellow of the Royal College of Physicians (Edinburgh). His early training in dermatology began following the award to him of an Anderson Travelling Fellowship in Medicine which allowed him to proceed to Paris, where he studied under Professors Levaditi and Gougerot at the Hôpital St Louis. He returned to Aberdeen in 1929, when he became Assistant Physician in Dermatology under the late Dr John Christie, and in 1931 he was appointed Senior Medical Officer in charge of the Skin Department and Physician in Skin Disease to the Aberdeen Sick Children's Hospital. From the inception of the National Health Service to 1968 he held the position of Consultant Dermatologist in Administrative Charge to the North Eastern Regional Hospital Board, and also Senior Lecturer in Dermatology in the University of Aberdeen. During the war years he served in the R.A.M.C. as Command Specialist (Dermatology and Venereology) and Scottish Command Specialist (Venereology) to the Central Mediterranean Force.

On his retirement in 1968, after a lifetime of service to dermatology, he became President of the British Association of Dermatology and he entertained the Association in Aberdeen. His contributions to dermatology were wide and varied and included a chapter in 'Modern Practice in Dermatology' on 'Disorders of the skin mainly affecting the hands and feet'. His papers included comments on a mild type of xeroderma pigmentosum. In 1955 he wrote on 'Erythrodermia with lipomelanic reticulum-cell hyperplasia of lymph nodes'. He was interested in the effect of antihistamines in itching and, as recently as 1969 in his retirement, wrote a paper for the *Practitioner* on 'Antihistamines'. He presented many unusual, instructive cases at meetings, and published reports of some, such as 'Linear basal-cell naevus', 'Connective tissue ossification presenting in the skin' and 'Myositis and myotonia in a case of multicentric reticulo-histiocytosis'. This last paper illustrates a little, his interest in medicine generally; his love of neurological problems was obvious to those who knew him well.

Tom Anderson was a modest man of deep religious conviction and an earnest churchman. He had always been actively associated with voluntary work for the elderly and his efforts in this direction were greatly extended when he retired. He was an expert fisherman and a connoisseur of wines. His interest in wines stemmed from his close association with France.

Although a man of quiet disposition he had a keen sense of humour and many will remember him as one of the generation of gentlemanly physicians who had little time to participate in more than their daily duties but, nevertheless, succeeded in doing so. He, single-handed at one time in his career, served the large north-east of Scotland area from Aberdeen to Inverness and beyond, including visits to the Shetland and Orkney Islands.

He is survived by his wife, a daughter and a son. We extend our sincere sympathy to them in their loss.

R.A. MAIN

Society Proceedings

Scottish Dermatological Society

President: PROFESSOR J.A.MILNE

The Annual General Meeting of the Scottish Dermatological Society was held at the Royal Infirmary, Edinburgh, on 25 and 26 May 1973. The following are case reports of two of the patients shown in the clinical section.

Pseudosarcoma (atypical fibroxanthoma) DR W.D.PATERSON (for PROFESSOR P.J.HARE)

A man aged 81, retired banker.

History. He has attended the Skin Department since 1969 for the treatment of senile keratoses of the scalp. His scalp had had considerable exposure to sunlight as he has been bald for 40 years and being fond of the sun, spends his holidays abroad. The keratoses have been treated with the curette, electrocautery, liquid nitrogen and 5-fluorouracil ointment, but never with X-rays.

In March 1973 he presented with a swelling which had arisen during the previous few months. It was painless and had not bled. It did not seem likely that it had appeared at the site of a previous keratosis.

On examination. On the left parietal area there was a smooth pink nodule approximately 1·5 cm by 0·75 cm.

Investigation. A biopsy was performed. The section (Fig. 1) showed a well circumscribed fibro-cellular mass occupying the whole depth of the dermis. It contained numerous multinucleated giant cells, some of whose cytoplasm was foamy. The mass was fairly vascular, and there were accumulations of lymphocytes peripherally.

Treatment. Conservative surgical removal was advised

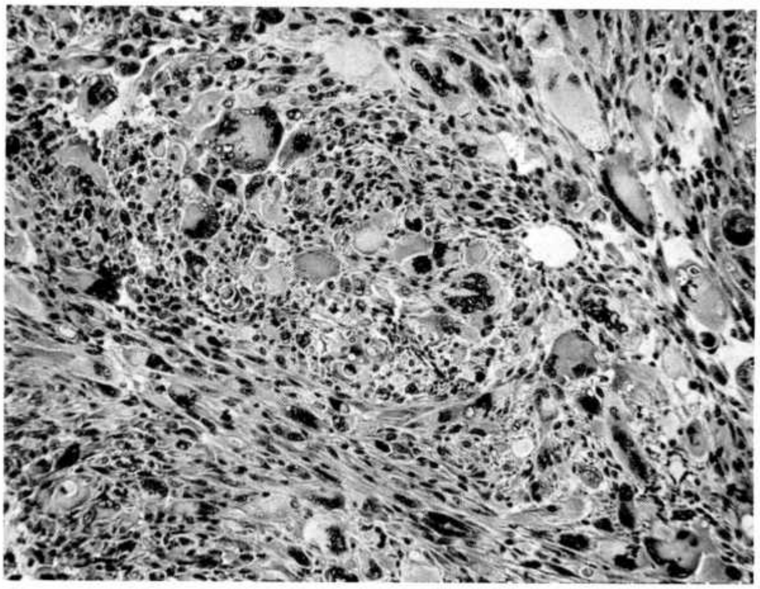

FIGURE 1.

Comment. This case appears to fall into the group of paradoxical or pseudo-sarcoma of the skin, affecting mainly elderly people with a history of long exposure to sunlight. This is the third case of this kind encountered here in the last three years.

REFERENCE

BOURNE, R.G. (1963) Paradoxical fibrosarcoma of skin: a review of 13 cases. *Medical Journal of Australia*, **1,** 504.

Primary Systemic Amyloidosis Dr J.A.SAVIN and Dr A.J.M.BUTT

A woman aged 73.

History. She was admitted to hospital following biopsy of thickened lips and gums which had caused her false teeth to fit badly over the previous 4 years. The oral lesions became alarmingly haemorrhagic, and the perioral skin was severely bruised.

There had been the following manifestations referable to amyloidosis. Decompression operations for a right and for a left carpal tunnel syndrome were performed at the ages of 66 and 67 respectively. A generalized bruising tendency was first noticed at the age of 66. Most marked on the fingers, even routine housework revealed it; these slowly developed soft bulbous tips (Fig. 1). At the age of 71, bleeding occurred from gelatinous perianal nodules which did not respond to routine injections for haemorrhoids. Intermittent diarrhoea and weight loss, at the age of 72, were suggestive of malabsorption. Mild angina pectoris had been present for a few months.

On examination. Translucent, yellow, oval lesions were noted on upper and lower eyelids. Bruises were present on the trunk and around the mouth. The mucous membranes inside the lips were occupied by haemorrhagic and friable masses. The tongue was not greatly enlarged although haemorrhagic nodules were present under the frenulum.

Investigations. Congo red staining demonstrated amyloid in biopsies of the lip, eyelid and rectum. There was no Bence-Jones' protein in the urine, no para-protein on serum electrophoresis, and no evidence of

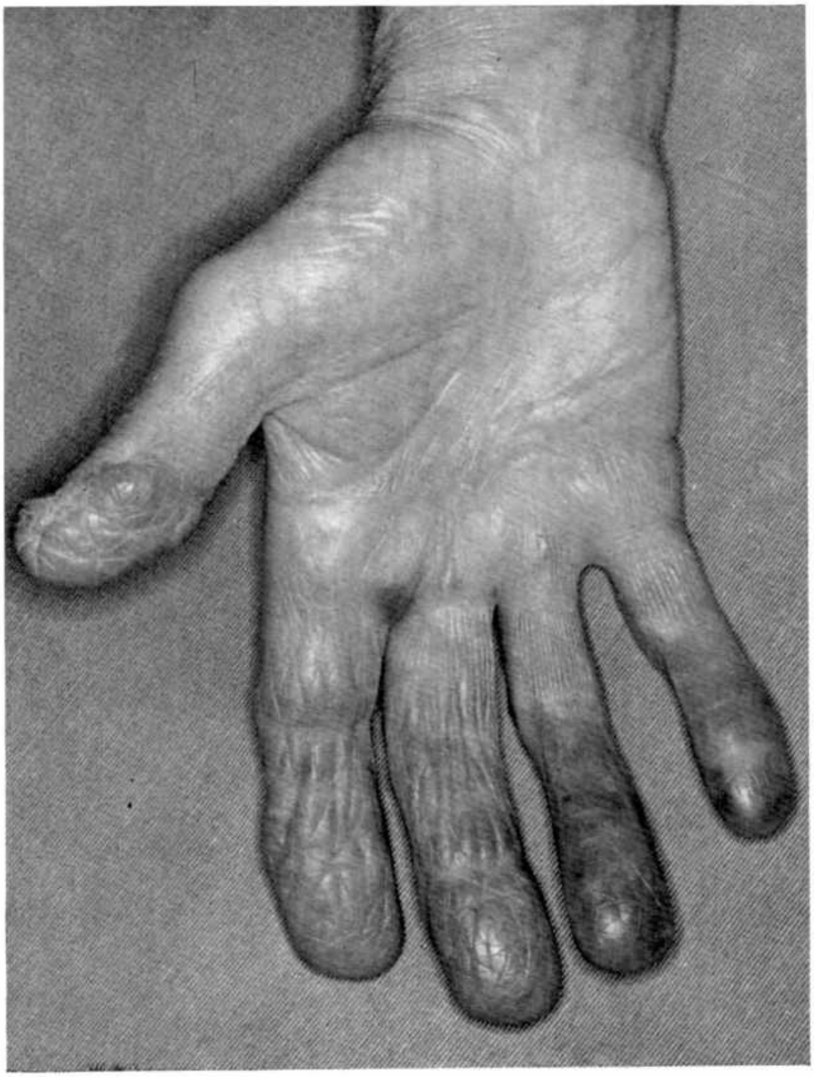

FIGURE I.

multiple myeloma in a radiological skeletal survey. A slight increase in plasma cells was found on bone marrow examination. Chest X-ray showed moderate cardiac enlargement, and the ECG was of low voltage. Coagulation tests, haemoglobin, white cell and platelet counts were normal. Tests for antinuclear factor, LE cells and the WR were negative. Blood urea was normal, proteinuria was absent, but creatinine clearance was slightly reduced.

Comment. The diagnosis of primary systemic amyloidosis is always difficult; in one series (Brandt, Cathcart & Cohen, 1968) symptoms were present on average for 3 years before the diagnosis was made. In our patient, symptoms preceded the diagnosis by 5½ years. Even so, we will keep the patient under review for myeloma, though there is no evidence for this at present. The diagnosis was made clinically from the unusual finger tip changes, which are strikingly like those described in detail for the first time by Levene (1969).

REFERENCES

BRANDT, K., CATHCART, E.S. & COHEN, A.S. (1968) A clinical analysis of the course and prognosis of forty-two patients with anyloidosis. *American Journal of Medicine*, **44**, 955.

LEVENE, G.M. (1969) Amyloidosis. *British Journal of Dermatology*, **81**, 234.

Book Reviews

A Plague of Corn. The Social History of Pellagra. DAPHNE A. ROE (1973) Ithaca and London: Cornell University Press. Pp. xxiii+217, illustrated. Price £5.50.

It was in 1735 that Dr Gaspar Casal, then practising in Oviedo in Spain, began to see patients afflicted with what they knew as 'mal de la rosa'. His book, 'Historia natural y medica de el Principado de Asturias', was published in 1762, three years after his death. The book is devoted to the endemic and epidemic diseases of Northern Spain: among the epidemic diseases he included 'mal de la rosa', which was the subject of the book's single illustration. During the eighteenth century the disease, under the Italian name of pellagra, was widespread throughout Italy and Spain, southwestern France, Austria, Roumania and the Turkish Empire. It was later recognized in South Africa, and by the early twentieth century it was disastrously common in the Southern United States.

In this remarkable book, Dr Daphne Anderson Roe presents a detailed history of pellagra. Too frequently so-called medical history consists of an anecdotal chronicle, often with emphasis on biographical irrelevancies. There is no disease the incidence and course of which are not influenced in some degree by social, and economic and hence by political factors. Conversely, many diseases have profound social consequences for individuals and communities. Even the incidence of those diseases in the aetiology of which genetic factors predominate, is influenced by marriage customs, by physical or cultural isolation, and by environmental factors which determine survival or early death. The quality and quantity of medical care in every country and every age have likewise been subject to broad social influences. Dr Roe appreciates to the full that the history of a disease can be effectively studied only in relation to man's total environment and she has succeeded brilliantly in demonstrating how this can and should be undertaken. She has covered comprehensively the international literature, medical, historical, economic, sociological and archaeological, and yet has contrived to present her material in an attractive, easily read narrative, in a simple flowing style, without jargon yet without condescension; the bibliographic tools of her scholarship are discreetly confined to some thirty pages of notes at the end of the book.

The history of pellagra has clear analogies with that of many other diseases, and lessons for the research worker in any field. With hindsight, the fallacies in many earlier hypotheses are obvious, yet to those who proposed these hypotheses they appeared to fit the available evidence, although in fact they did not do so; enthusiasm leads the unwary investigator to overlook findings which do not support his conception of the truth.

The clinician who is not yet convinced of the value of the historical approach will nevertheless wish to consult this volume for its critical assessment of the present state of our knowledge of pellagra and of some of the unsolved problems of a disease which man has the means but apparently not always the will to eliminate, for it is still prevalent in some parts of the world.

ARTHUR ROOK

Practice of Dermatology. P.N.BEHL (1972) 2nd Edition. Delhi: Oxford Printcraft India Ltd, in association with Arnold-Heinemann, India. Pp. 528, 65 pages of black and white illustrations, 1 page in colour. Price £3.00.

Ten years have passed since the publication of the first edition of Professor Behl's textbook. It was written for students and general practitioners and was highly successful, both in its English version and in a subsequent Hindi translation. Those who are fortunate enough to know some of the leaders of contemporary Indian dermatology will be aware of the magnitude of Dr Behl's contribution to the rising standards of teaching and practice of the specialty in India. Dr Behl has inspired the creation of the Skin Institute of Delhi; the Trustees of the Institute have subsidized the publication of the present volume, making possible the remarkably low price.

In preparing his second edition Dr Behl has enlisted the collaboration of eighteen colleagues as contributors or advisers; these include Chaim Berlin of Israel, Leeming and Loewenthal of South Africa and fifteen of India's most distinguished dermatologists. It is evident from the success of the original editions in English and in Hindi that this book satisfies a need. The scope of most chapters is admirably suited to the purpose for which they are intended. A general criticism of many chapters, however, is that too much space has been given to rare disorders, even to some said not to occur in India; the confusing account of the erythrodermas actually includes that very dubious entity 'Savill's disease'. The consequent need to limit the space allotted to common diseases accounts for a number of potentially misleading generalizations, for example—'the majority of hereditary diseases are incurable'. Important recent work which should surely have been included, since it was well established long before the date at which this edition presumably went to press, includes the epidemiologically highly significant studies on the two types of *Herpesvirus hominis* and the two types of wart virus. Intestinal lesions in dermatitis herpetiformis are surely worthy of full discussion. The least satisfactory chapter is that on 'Anatomy, Physiology, Embryology, Bacteriology and Pathology'. The author was heroic to attempt so much in 15 pages, and cannot be blamed for some confusing omissions. The editor should, however, not have allowed to pass unchallenged the remarkable (undocumented) statement: 'Scalp hair is under the influence of oestrogen, beard hair under androgen and pubic and axillary hair under gonadotrophins'.

Therapeutic recommendations are in general sound and orthodox, but is it really desirable to curette the lesions of orf? The current reaction in Europe and the U.S.A. against the use of radiotherapy in benign dermatoses has probably been too strong, but can one reasonably claim that 'X-ray therapy is a good stand-by for resistant cases of tinea pedis'?

There are now so many Indians permanently resident in Britain that most dermatologists in this country see many Indian patients. Understandably, immigrants retain many of their own customs. In those parts of Britain with large Indian communities, indigenous Indian remedies and cosmetic preparations are commercially available and are widely used. Since some of these substances may cause dermatitis, the pages of this book on contact dermatitis may provide British dermatologists with practical information not otherwise readily available. It would, however, have been interesting to be told more about the composition of these preparations or at least the botanical names of the plant species from which they are derived.

The numerous black and white illustrations are not all of high quality, but the majority of them are good enough to be informative. The book ends with a four page bibliography of textbooks on most aspects of dermatology.

In reading this book one cannot fail to be impressed by the sincerity of Dr Behl's purpose and the warm humanity of his concern for his patients as people. Perhaps in future editions he may find it possible to impose his editorial policy more firmly, and to raise the quality of the poorer chapters to that of the best.

ARTHUR ROOK

British Journal of Dermatology (1974) **90**, 364.

News and Notices

Third Rumanian National Congress of Dermatology

This Congress will be held in Bucharest from 11 to 13 September 1974. The main themes of the Congress are Cutaneous Reticulosis, and Gonococcal and Non-gonococcal Urethritis. There will also be a clinical meeting. Information about the Congress may be obtained from Professor P. Vulcan, Secretariat of the Third National Congress of Dermatology, 8–10, Progresului st Bucharest, Rumania.

A New Dermatological Journal—*Acta dermatovenereologica Iugoslavica*

The first issue of this new journal of dermatology has just been published. The papers, which cover a wide range of clinical and research topics, are in Serbo-Croat but are provided with adequate summaries in English. The Editors invite contributions in English, for which Serbo-Croat summaries will be provided. The Editor-in-Chief is Dr J. Fettich and the Editor is Dr A. Kansky. The address of the Editorial Board is 61000 Ljubljana, Komenskega ul. 4, Jugoslavia.

APPOINTMENTS

St Thomas' Hospital, London

Dr Martin Munro Black has been appointed Consultant Dermatologist to St Thomas' Hospital, London. Dr Black qualified M.B., B.S. at Durham University, Newcastle-upon-Tyne, in June 1963. He became M.R.C.P. (London) in April 1967 and proceeded to the M.D. in December 1970. In his post-graduate career he was awarded the Dowling Travelling Fellowship, in 1971–72, and a Fulbright Travelling Scholarship, 1972. He started dermatology as a Registrar in the Royal Victoria Infirmary, Newcastle-upon-Tyne, in September 1966, and he came to St John's Hospital, London, as a Senior Registrar and Tutor in October 1968. He became Senior Registrar and Tutor in Histopathology of the Skin at the Institute of Dermatology, London, in January 1970. His main publications have been related to skin collagen and thickness, and he has had many papers published on this subject. He has always been interested in histopathology, and has paid particular attention to lichen amyloidosus, pityriasis lichenoides and lichen planus.

Worthing, Southlands and District Group, Chichester and Graylingwell Group and the Redhill and Netherne Group

Dr Ashley Vincent Levantine has been appointed Consultant Dermatologist to the Worthing, Southlands and District Group, the Chichester and Graylingwell Group and the Redhill and Netherne Group of Hospitals. Dr Levantine qualified M.B.B.S., M.R.C.S., L.R.C.P., from the London Hospital, in 1966, having obtained the James Anderson Prize in Elementary Clinical Medicine. He worked in general medicine until November 1970, when he became Registrar in Dermatology at the Whipps Cross Forest Group, and in July 1972 he became Senior Registrar at the Middlesex Hospital. His published work includes papers on the immunology of psoriasis, and viral studies in toxic erythema.

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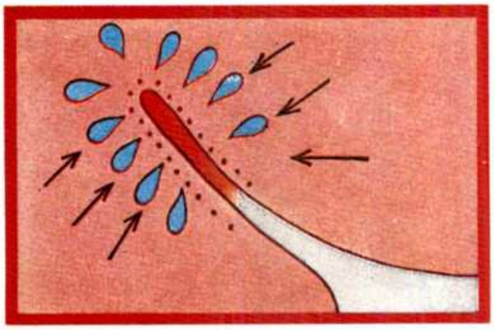

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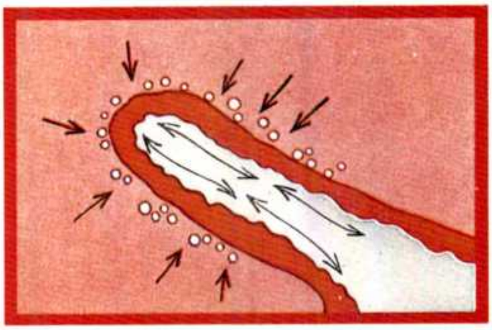
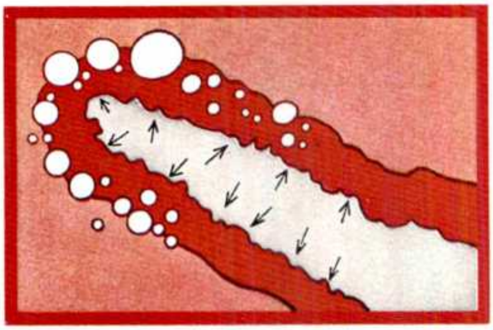
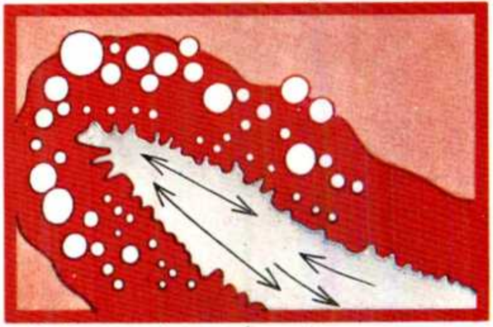


maceration and chafing



secondary infection



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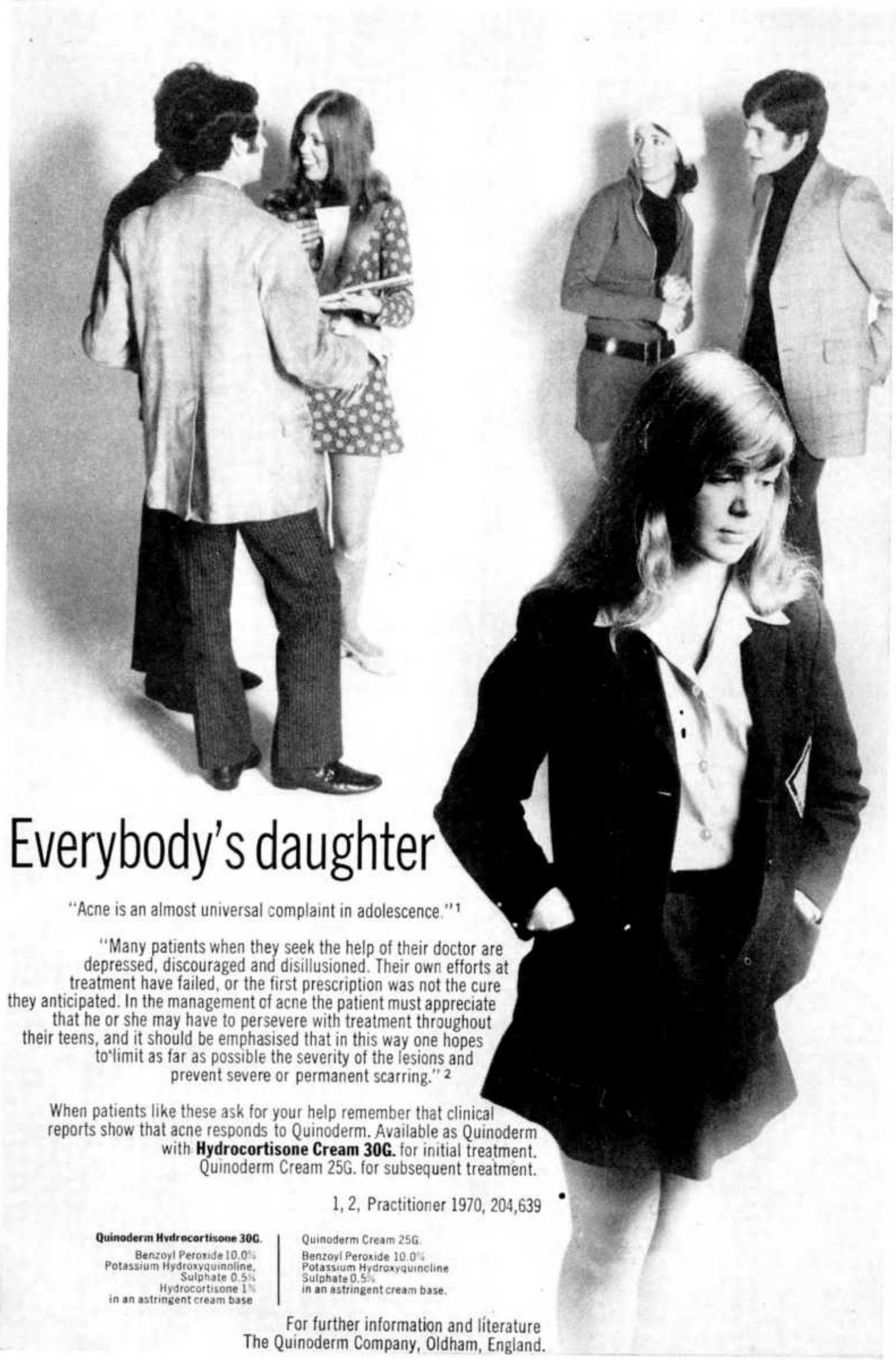

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Volume 1, Number 1, January 1974

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R.G. Sampson, G.C. Scroop and W.J. Louis

Influence of pyrophosphate and diphosphonates on rat liver adenylate cyclase
J.A. Eisman, T.J. Martin, R.Pilczyk, D.G. Legge and H.S. Sutcliffe

A microperfusion study in the rat of the permeability of the papillary segments of the nephron to ^{24}Na
T. Morgan

The haemodynamic effects of quazodine, a cardiac stimulant, in experimental *E. coli* endotoxin shock in the cat
J.R. Parratt and Eileen Winslow

A comparison of the activities of the β-adrenoceptor agonists MJ9184–1 and (—)- isoprenaline in guinea-pig and cat preparations
T. Davey, E. Malta and C. Raper

Potentiation of an effect of allopurinol on pyrimidine metabolism by chlorothiazide in man
Margaret H. Wood, W.J. O'Sullivan, M. Wilson and D.J. Tiller

Cardiovascular responses produced by the injection of isoprenaline and propranolol into the cerebral ventricles of the unanaesthetized dog
E.L. Conway and W.J. Lang

'Steady-state' properties of the baroreceptor-heart rate reflex in essential hypertension in man
P.I. Korner, M.J. West, J. Shaw and J.B. Uther

Coronary haemodynamic effects of nifedipine (*Bay a 1040*) and glyceryl trinitrate in unanaesthetized dogs
S.E. White, W.L. Porges and R.J. McRitchie

An antihypertensive action of propranolol in DOCA/salt-treated rats
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Index Vol. 147, No. 4, 1973

Haustein, U.-F. (Berlin): Tubuläre Strukturen in kranken und scheinbar gesunden Nieren bei chronischem und viszeralem Lupus erythematodes. Eine elektronenmikroskopische Studie
(Tubular Structures in Affected and Apparently Normal Kidneys in Chronic Discoid and Systemic Lupus erythematosus. An Electron Microscopic Study)

Malten, K.E.; Kuiper, J.P., and *van der Staak, W.B.J.M.* (Nijmegen): Contact Allergic Investigations in 100 Patients with Ulcus Cruris . . . .

Eleuterio, M.K.; Grappel, S.F.; Caustic, C.A., and *Blank, F.* (Philadelphia, Pa.): Role of Keratinases in Dermatophytosis. III. Demonstration of Delayed Hypersensitivity to Keratinases by the Capillary Tube Migration Test . . . .

Haim, S.; Shafrir, A.; Haim, N., and *Lichtig, C.* (Haifa): Microangiopathy in Cases of Granuloma annulare . . . .

Bhutani, L.K. and *Deshpande, S.G.* (New Delhi): Erythropoietic Protoporphyria. A Case Report . . . .

Pasricha, J.S. and *Nayyar, K.C.* (New Delhi): Fox-Fordyce Disease in the Post-Menopausal Period Treated Successfully with Electrocoagulation . . . .

Günther, S. and *Alston, W.* (Glasgow): Follicular Keratoses.
Pilot Studies of Serum Level of Vitamin A and Liver Function Tests during Administration of Retinoic Acid in Hyperkeratosis follicularis et parafollicularis (Kyrle's Disease), Pityriasis rubra pilaris, and Keratosis follicularis (Darier's Disease) . . . .

Šalamon, T.; Lazović, O., and *Milicević, M.* (Sarajevo): Histologic Findings in Angioma senile. The Phenomenon of Epidermo-Dermal Surveillance . . . .

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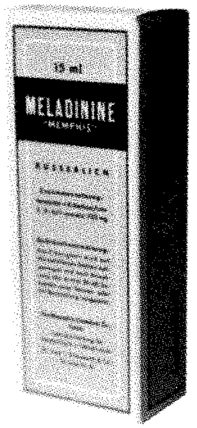

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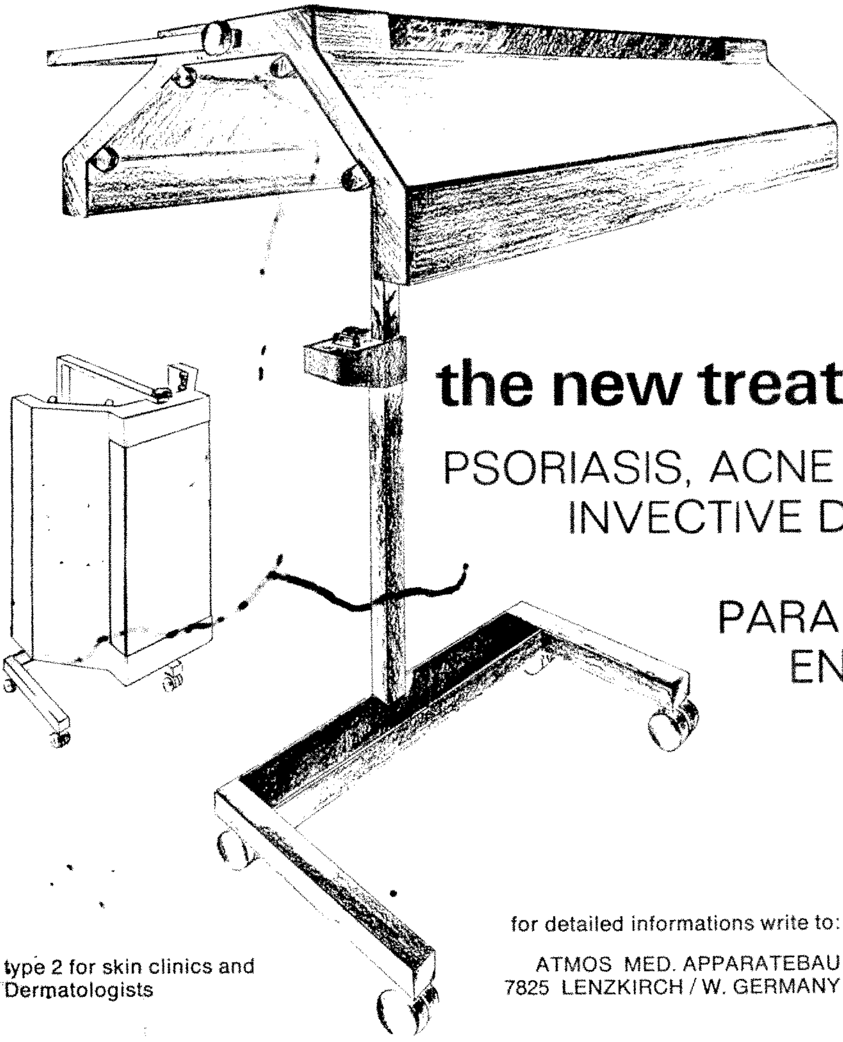

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Textbook of Dermatology

Edited by
Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and
F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

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British Journal of Dermatology, Volume 90, Number 3, March 1974

CONTENTS

PAGE

Clinical and Laboratory Investigations

A Clinical Syndrome Characteristic of Primary Type IV–V Hyperlipoproteinaemia 245
PETER BORRIE AND JOAN SLACK

The Association of Familial Hyperbetalipoproteinaemia with Waardenburg's Syndrome 255
MOHAMMED AMER AND MOHAMMED EL-SHAZLY

The Response of the Sweat Glands to β-Adrenergic Stimulation with Isoprenaline 263
J. A. WARNDORFF AND M. HAMER

Onset of Palmar Sweating in Newborn Infants 269
JULIAN VERBOV AND JANE BAXTER

Sweat Responses of a Hyperhidrotic Subject 277
JUDITH A. ALLEN, JANET E. ARMSTRONG AND I. C. RODDIE

Changes in the T-wave of the Electrocardiograph in Urticaria 283
Y. SHOENFELD AND S. SCLAROVSKY

Heat Urticaria 289
M. W. GREAVES, I. B. SNEDDON, A. K. SMITH AND D. R. STANWORTH

Granuloma Pyogenicum—A Hitherto Unrecognized Complication of Smallpox Vaccination 293
ISMAIL ZAYID AND SAMIR FARRAJ

A Dermatosis Specifically Associated with a Tumour of Pancreatic Alpha Cells 301
R. D. SWEET

Blood and Pustule Culture in Pustular Psoriasis 309
MUNA MATTA

Reduced Activation of Epidermal Phosphorylase in the Lesions of Psoriasis 313
P. D. MIER AND JOSE J. M. A. VAN DEN HURK

Pharmacology and Treatment

Combined 8-Methoxypsoralen and Black Light Therapy of Psoriasis. Technique and Results 317
GERHARD WEBER

Axillary Hyperhidrosis. Its Quantification and Surgical Treatment 325
DOWLING D. MUNRO, JULIAN L. VERBOV, DAVID J. O'GORMAN AND ANTHONY DU VIVIER

Topical Antibiotics in Pyodermas 331
S. T. ZAYNOUN, M. T. MATTA, M. M. UWAYDA AND A. K. KURBAN

Cryoprobe Treatment of Acne Conglobata 335
JAMES J. LEYDEN, OTTO H. MILLS AND ALBERT M. KLIGMAN

Case Reports

Intra-epidermal Epithelioma. Report of Case with Invasion After Many Years 343
PETER BERGER AND RICHARD BAUGHMAN

Cutaneous Leiomyomata in Two Brothers 351
K. C. VERMA, S. D. CHAWDHRY AND K. S. RATHI

Comment

New Concepts in the Management of Mycosis Fungoides 355
ZVI FUKS, MALCOLM A. BAGSHAW AND EUGENE M. FARBER

Obituary

Thomas Edge Anderson 358

Society Proceedings

Scottish Dermatological Society, Annual General Meeting, Edinburgh, 25 and 26 May 1973 359

Book Reviews ...... [illegible]

News and Notices 364

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19.8.85

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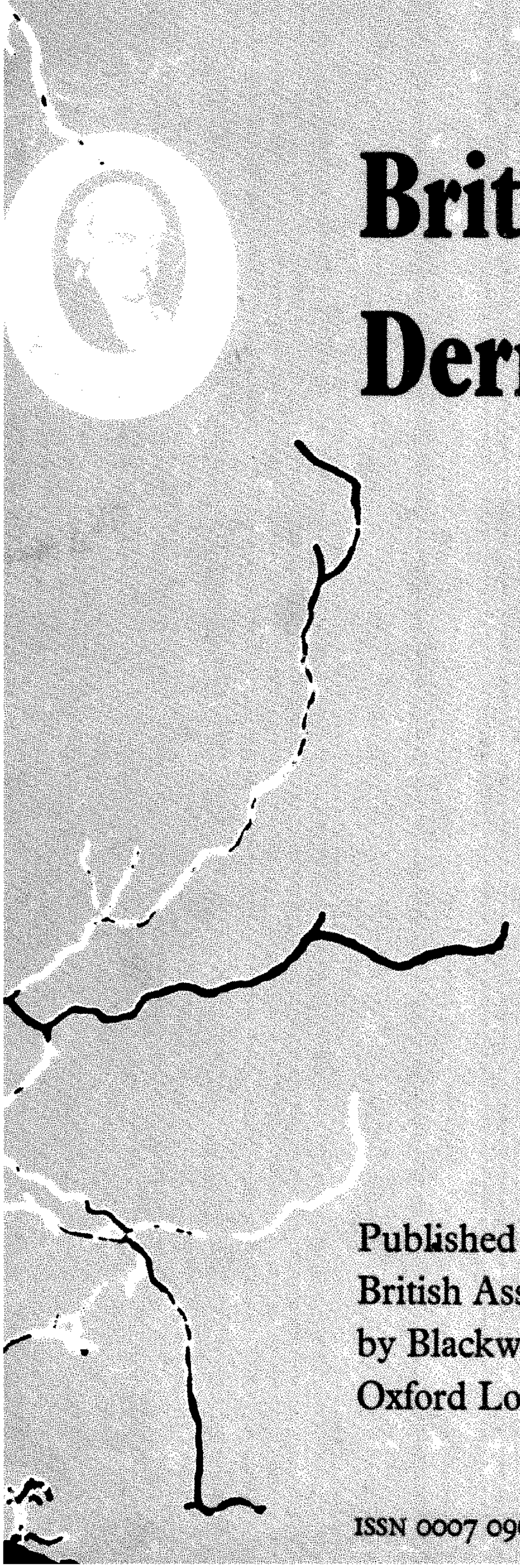
Volume 91, Number 1, July 1974

British Journal of Dermatology



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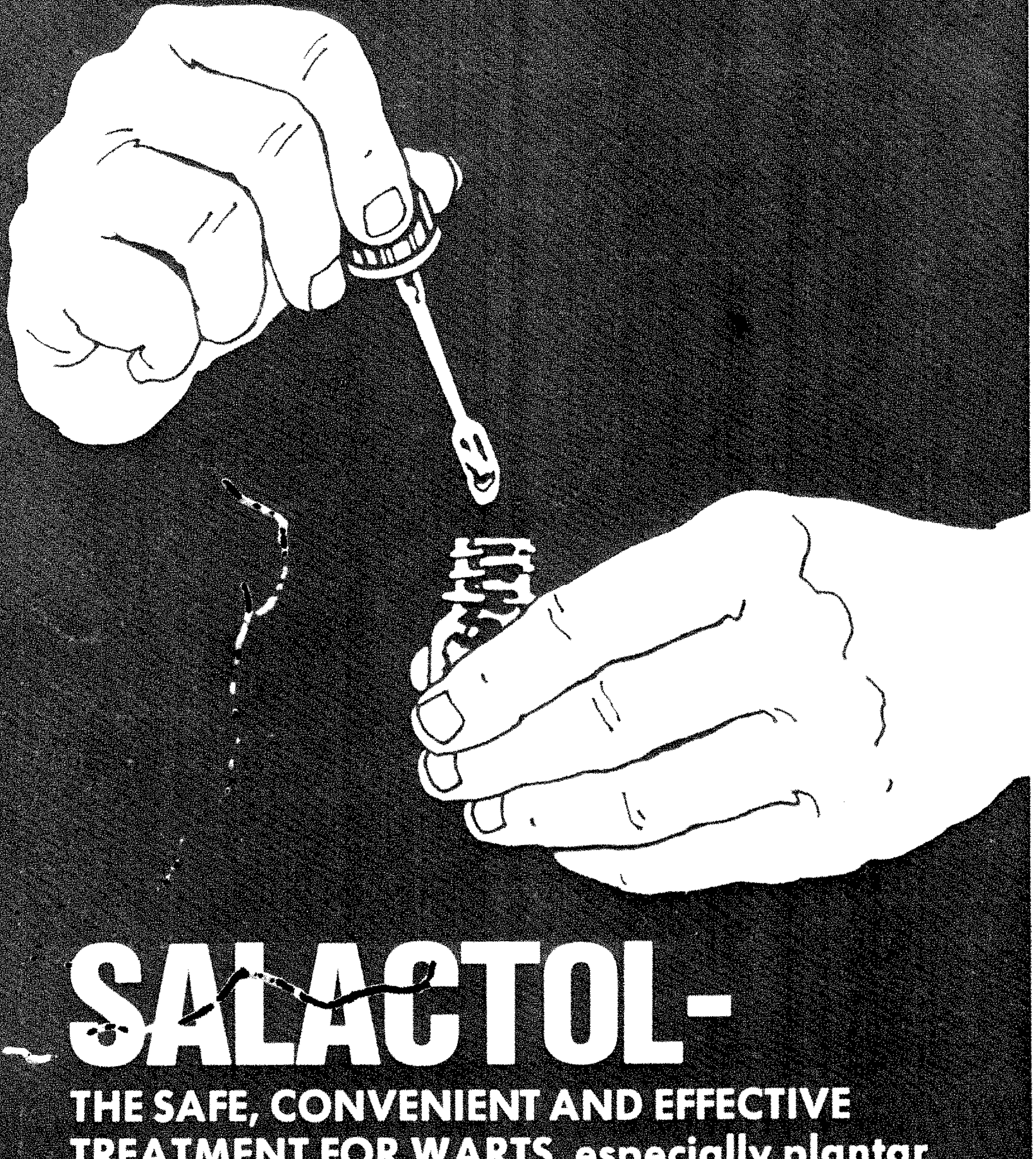

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Volume 1, Number 2, March–April 1974

Drug-induced changes in blood flow in the acutely ischaemic canine myocardium; relationship to subendocardial driving pressure
R.J. Marshall and J.R. Parratt

Actions of dantrolene solution contractions of the tibialis anterior and soleus muscles of cats under chloralose anaesthesia
M.W. Nott and W.C. Bowman

The role of pre- and post-junctional cholinoceptors in the action of neostigmine at the neuromuscular junction
Sandra N. Webb and W.C. Bowman

Excitatory and inhibitory effects of noradrenaline on the isolated guinea-pig vas deferens
R.M. Wadsworth

Visual evoked potentials as a measure of drug effects on arousal in the rabbit
H. Goldberg, T.B. Horvath and R.A. Meares

Plasma renin response to acute blockade of angiotensin II in the anaesthetized rat
Helen F. Oates, Gordon S. Stokes and Robyn G. Glover

Plasma renin activity of rats and rabbits immunized against angiotensin II
Helen F. Oates and Gordon S. Stokes

Short communications

The renin–aldosterone systems in the pregnant rhesus monkey (*Macaca mulatta*)
E. Marilyn Wintour, E. Knobil, B.A. Scoggins, S.L. Skinner and J.P. Coghlan

A novel sequence in kangaroo angiotensin I
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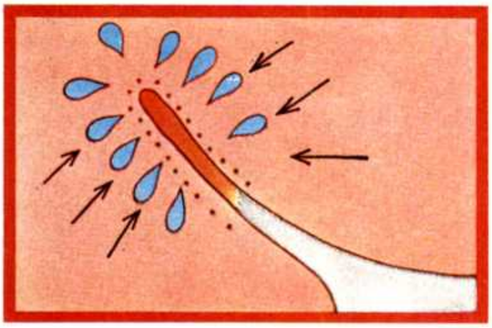

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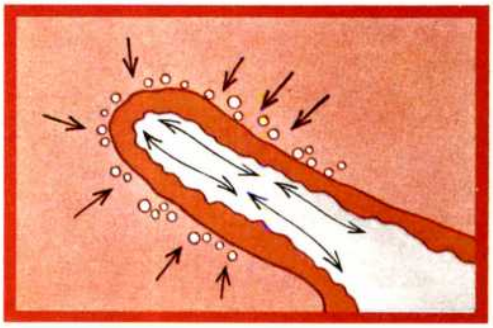

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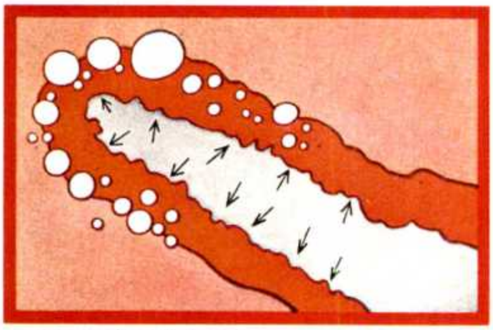

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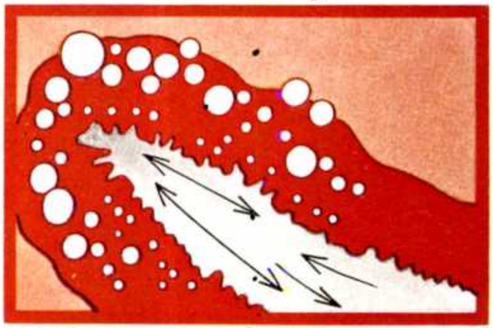

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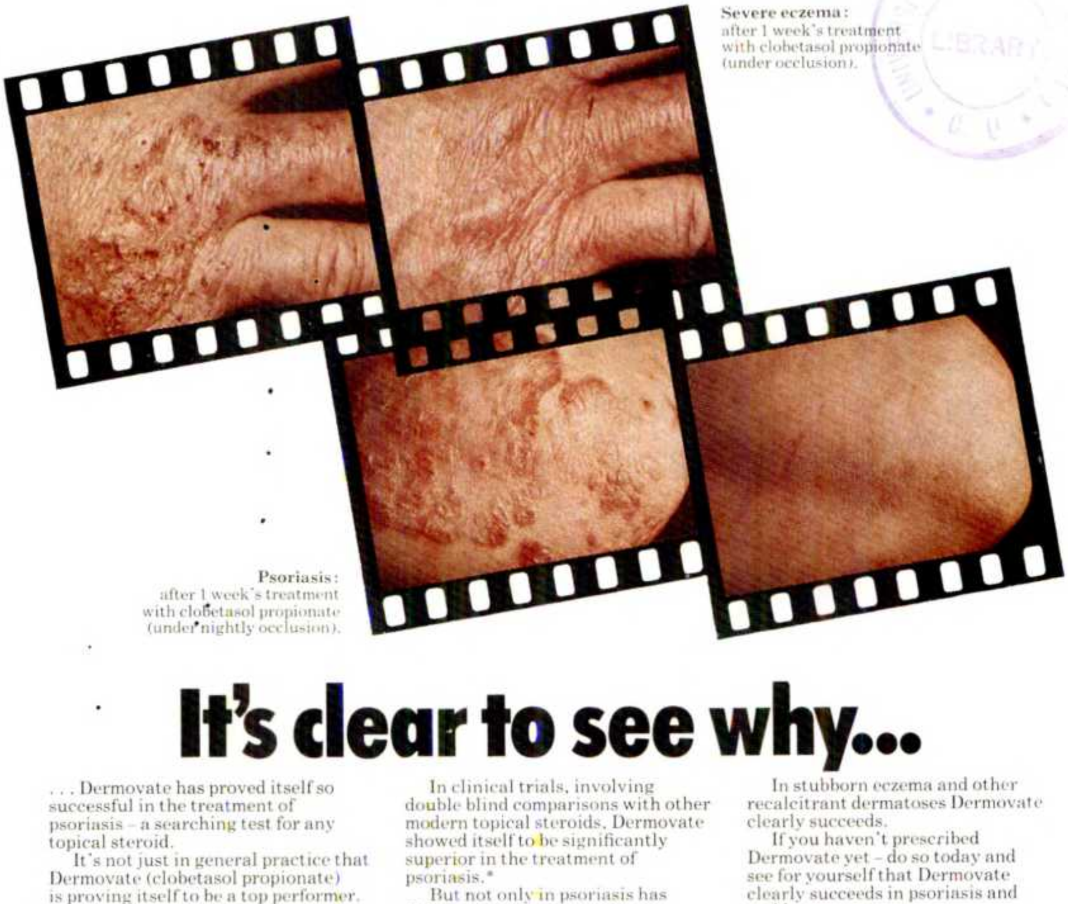

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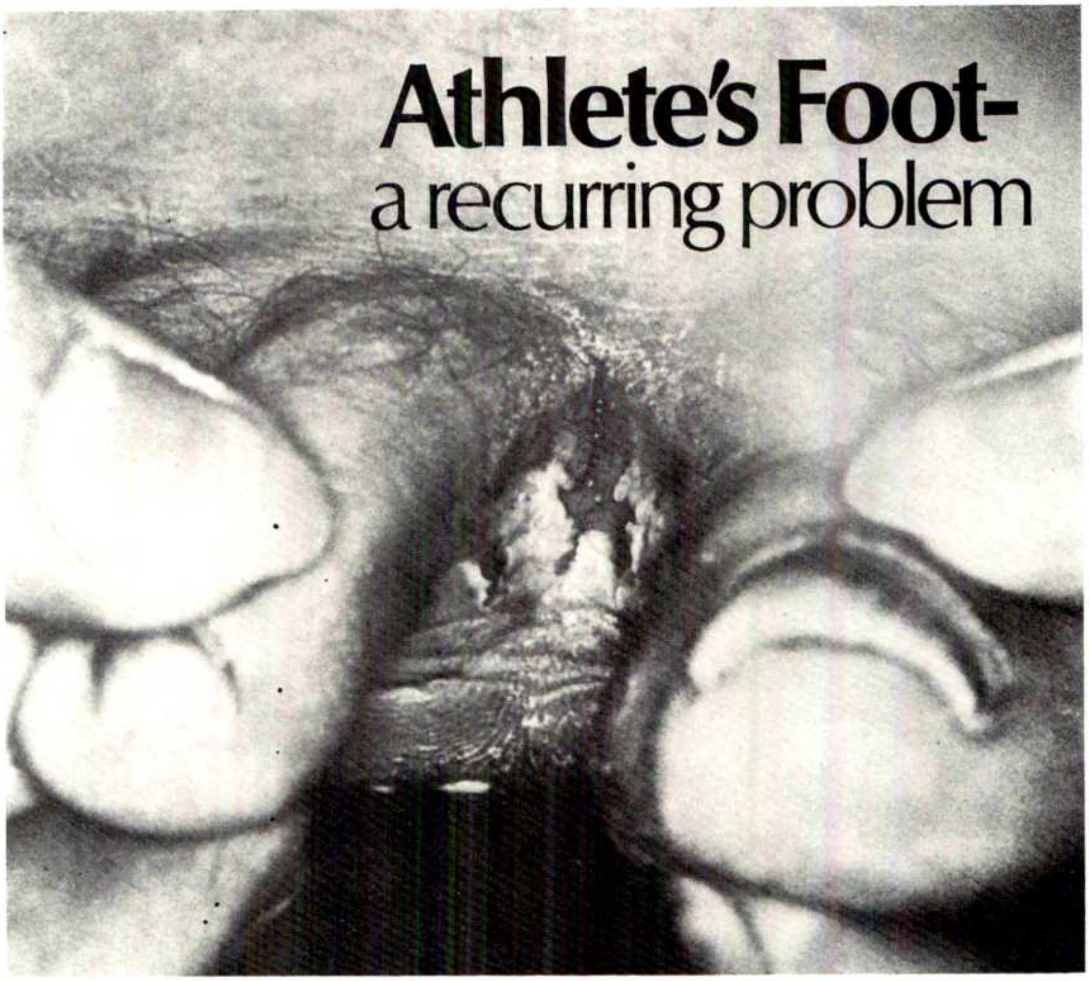

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Clinical and Laboratory Investigations

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Leukocyte migration inhibition in progressive systemic sclerosis

P. HUGHES, SHIRLEY HOLT AND N.R. ROWELL

The University Departments of Dermatology and Pathology, The General Infirmary at Leeds, Leeds LS1 3EX

Accepted for publication 4 October 1973

SUMMARY

The leukocyte migration test was used to examine patients with progressive systemic sclerosis for evidence of cell-mediated immunity to a wide range of autologous, homologous and heterologous antigens. Ten patients with progressive systemic sclerosis and seventeen normal controls were tested for sensitivity to autologous lymphocytes, microsomal and mitochondrial fractions of human cadaver liver, human myelin basic protein and porcine thyroglobulin. Migration indices in the control group showed a uniform response with, in general, only minimal reactivity for each of the antigens tested. By contrast, the patients with progressive systemic sclerosis showed a significantly greater degree of migration–inhibition using autologous lymphocytes, the liver microsomes and mitochondria, and the myelin basic protein. Leukocyte migration in the presence of porcine thyroglobulin did not differ, however, from that in the normal controls. The findings indicate that there is in progressive systemic sclerosis, widespread cell-mediated hypersensitivity, much of which is directed against both autologous and homologous antigens.

The aetiology of progressive systemic sclerosis (PSS) remains uncertain, but the multi-system nature of the disease with alterations in connective tissue and with vasculitis (Rodnan, 1963), the high incidence of autoantibodies (Beck *et al.*, 1963), the occasional association with Sjögren's syndrome and the clinical 'overlap' with systemic lupus erythematosus (SLE) (Tuffanelli & Winkelmann, 1962), all suggest that immunological mechanisms may be involved in its pathogenesis. The development of an autoimmune lesion in an experimental situation may require the presence of both humoral and cell-mediated immune responses (Brown, Glynn & Holborrow, 1967), and the leukocyte migration test has been shown to provide *in vitro* evidence of such cellular sensitization both in animals (Krohn & Finlayson, 1973) and in man (Søborg & Bendixen, 1967; Federlin *et al.*, 1971). The present report describes further investigations in PSS, using this test to detect cell-mediated immunity to a range of antigens prepared from autologous, homologous and heterologous tissues.

MATERIALS AND METHODS

Patients

Ten patients with PSS, one of whom was male, with a mean age of 52·2 ± 13·0 years, were investigated.

All the patients presented with Raynaud's phenomenon and later developed acrosclerosis and systemic involvement. The duration of the disease, taken from the onset of the Raynaud's phenomenon, varied from 3 to 26 years, with a mean of 12·8 years. Antinuclear antibodies were detected in eight of the group. Only two of the patients were receiving corticosteroids (in a dose of 5–10 mg prednisone per day) at the time the tests were performed. Controls consisted of seventeen normal, healthy volunteers, four of whom were male, with a mean age of 35·8 ± 12·1 years.

Preparation of antigens

Autologous lymphocytes were prepared from blood anticoagulated with 4% ethylenediaminetetra-acetic acid (1 part in 10) by centrifugation on a 'Ficoll-Triosil' gradient (Evans & Middleton, 1970). After washing with TC199 (Wellcome Reagents Ltd), the lymphocytes were lysed by freezing and thawing, and the equivalent of 5×10^5 cells added as antigen to each migration chamber.

Mitochondrial-rich and microsomal-rich fractions of human cadaver liver, obtained within 3 h of death, were prepared by the method of Hogeboom (1955). The resulting fractions were added to migration chambers to give final concentrations of 800 μg protein/ml and 400 μg protein/ml respectively.

Human myelin basic protein, prepared by the method of Eylar *et al.* (1969) and migrating as a single band on polyacrylamide gel electrophoresis, was kindly donated by Dr J.Dickinson, Department of Pathology, University of Leeds, and used at a final concentration of 300 μg protein/ml in the migration chambers.

Porcine thyroglobulin (Sigma Chemical Company) was used at a concentration of 300 μg protein/ml in the migration chambers.

Leukocyte-migration test

The method used was based on the micro-modification devised by Federlin *et al.* (1971). Thirty ml of venous blood were anticoagulated by defibrination, and the erythrocytes then sedimented by adding 5 ml of 'Plasmagel' (Laborataire Roger Bellon, Neuilly, France). The supernatant leukocyte-rich plasma was pipetted off, centrifuged at 100 *g* for 5 min, and the resulting cell pellet was resuspended and washed three times in TC199. The washed leukocytes were finally suspended in three volumes of TC199, aspirated into 10 μl capillary tubes (Shandon Scientific Ltd) which were then sealed and centrifuged at 50 *g* for 5 min. Each capillary tube was broken cleanly at the fluid-cell interface and then mounted with silicone grease in plastic migration chambers (Sterilin Ltd) containing TC199 enriched with 20% foetal bovine serum (Wellcome Reagents Ltd) and appropriate antigen. The chambers were sealed by silicone greased cover slips and incubated at 37°C for 22–24 h. All antigens were tested in quadruplicate. The extent of leukocyte migration was measured by weighing the projected image of the migrating cells on a standard weight card. The extent of migration in the presence of antigen was expressed as a ratio (the 'migration index') of the migration occurring in control cultures.

RESULTS

The extent of leukocyte migration–inhibition found in the patients with PSS and in the controls is shown in Fig. 1. The control group showed a remarkably uniform pattern of response to each of the five antigens tested, with mean migration indices that only varied between 0·84 ± 0·22 and 0·93 ± 0·17. A number of individual control migration indices, however, did show some evidence of inhibition with each of the antigens examined. Such a response of normal controls to high concentrations of

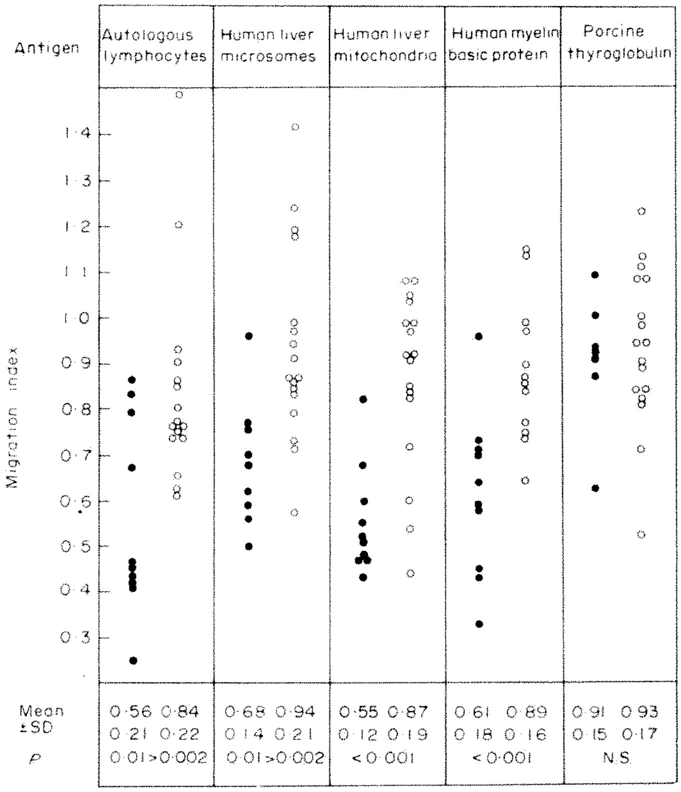

FIGURE 1. Leukocyte migration–inhibition in patients with PSS and controls. ●, PSS group; ○, control group.

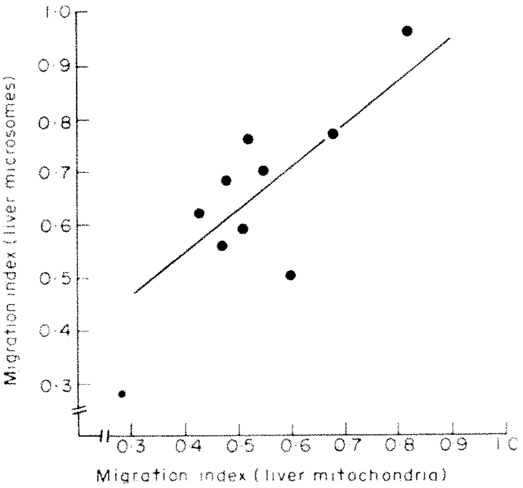

FIGURE 2. Correlation between the leukocyte migration–inhibition produced by human liver mitochondria (800 μg/ml) and human liver microsomes (400 μg/ml) in patients with PSS. $r = 0{\cdot}72$; $t = 2{\cdot}72$; $0{\cdot}05 > P > 0{\cdot}02$.

antigens, particularly when prepared from homologous tissues, has been noted in other investigations (Miller *et al.*, 1972; Calder *et al.*, 1972) and may be related to the migration inhibition found by Weir & Suckling (1971) in normal guinea-pigs.

The patients with PSS showed, by way of contrast, a very significantly greater degree of leukocyte migration–inhibition with four of the five antigens examined, i.e. the autologous lymphocytes, the microsomal and mitochondrial fractions of human liver and the myelin basic protein. The mean leukocyte migration–inhibition with all these four antigens (mean migration indices of $0{\cdot}55 \pm 0{\cdot}12$ to $0{\cdot}68 \pm 0{\cdot}14$) was again reasonably uniform and in marked contrast to the reaction to porcine thyroglobulin (migration index of $0{\cdot}91 \pm 0{\cdot}15$) which did not differ significantly from the response of the normal control group (migration index of $0{\cdot}93 \pm 0{\cdot}17$).

The correlation between the leukocyte migration–inhibition produced by human liver mitochondria and microsomes, myelin basic protein and autologous lymphocytes was also examined. Fig. 2 shows that there was a significant correlation between the leukocyte migration–inhibition produced by liver microsomes and mitochondria ($r = 0{\cdot}72$; $t = 2{\cdot}72$; $0{\cdot}05 > P > 0{\cdot}02$). No correlation was found between mitochondria and myelin basic protein or lymphocytes, or between microsomes and myelin basic protein.

DISCUSSION

This investigation has revealed a high incidence of significant leukocyte migration–inhibition to a wide range of autologous and homologous tissue antigens in patients with PSS. The leukocyte migration test is an *in vitro* indicator of cell-mediated immunity (Søborg & Bendixen, 1967) and the pattern of sensitization to tissue antigens in PSS has proved to be similar to that found in an earlier study of SLE (Hughes, Holt & Rowell, 1974). This similarity, we feel, is not too surprising in view of the fact that both disorders have in common a high incidence of humoral auto-antibodies (Beck, 1963). The possibility that this wide spectrum of sensitization is due to cross-reactivity or sharing of a few common antigens seems unlikely in view of the generally poor correlation shown between the different 'antigen' preparations. While contamination could account for the correlation shown between liver mitochondria and microsomes, the poor correlation between 'antigens' as diverse as lymphocytes, liver microsomes and pure myelin basic protein, suggests rather the operation of multiple specific sensitivities.

The significance of leukocyte migration–inhibition in response to tissue antigens, in terms of the actual pathogenesis of a disease, remains uncertain. Cellular sensitization to tissue antigens has now been reported in Addison's disease (Nerup, Andersen & Bendixen, 1970), Sjögren's syndrome (Berry, Bacon & Davis, 1972), Hashimoto's disease (Calder *et al.*, 1972), SLE (Hughes *et al.*, 1974) and both primary biliary cirrhosis and chronic active hepatitis (Miller *et al.*, 1972); the sensitization in these last two diseases possibly being to a liver specific antigen. Moreover, in experimental canine gastritis, the development of the gastric lesion is closely associated with the appearance of leukocyte migration–inhibition using the gastric juice as immunizing antigen (Krohn & Finlayson, 1973). In all these instances, the test may well be an *in vitro* indicator of the presence of pathogenetically important cellular autosensitization. Leukocyte migration–inhibition with mitochondria, however, has also been described after both myocardial infarction and surgery (Wartenberg & Brostoff, 1973), and could then be analogous to the transient and usually low titre autoantibody production that may also develop after similar non-autoimmune tissue damage (Hutchinson, Patel & McArthur, 1971).

The presence of cellular sensitization to such a wide range of tissue antigens in PSS seems more indicative of a loss of tolerance, as we suggested in SLE (Hughes *et al.*, 1974), than to be simply the result of a secondary reaction to tissue damage and inflammation. Such a loss of tolerance on the part of presumptive thymus-dependent (T) lymphocytes could facilitate autoantibody production by non-thymus-dependent (B) lymphocytes (Allison, Denman & Barnes, 1971) and account for the emergence of the lymphocyte cytotoxicity described by Currie, Saunders & Knowles (1971) in patients with PSS. There have been many theories as to the aetiology of PSS with, in the last few years, an increasing amount of evidence that many patients, and their symptomless relatives, have immunological abnormalities (Fennell, MacLachlan & Rodnan, 1962; Rodnan, 1963; Currie *et al.*, 1971). Our finding of widespread cell-mediated hypersensitivity in PSS to human tissue antigens adds further weight to the growing impression that immunological factors are involved in the pathogenesis of the disease.

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A 7-year follow-up study of ninety patients with psoriasis

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SUMMARY

Of 142 patients with psoriasis who attended a dermatological clinic in 1966, ninety were interviewed and re-examined 7 years later. Twenty-eight patients (29%) had been treated at home, fifty-eight (64%) had been treated with the Ingram regime as out-patients, and sixteen (17%) had been admitted.

It was concluded that those treated as out-patients had a longer duration of remission than those treated at home. In these out-patients, the only significant indicator of the outcome was the type of psoriasis at presentation. Those presenting with guttate psoriasis had a better prognosis.

Most papers on the subject suggest that patients with psoriasis treated in a dermatology department will almost certainly have their lesions cleared or improved, but that the likelihood of relapse is very high. Lane & Crawford (1937) found that only 27·7% of 231 cases had a complete remission, the average duration of this being 3·1 months. In Church's survey (1958), 74% of 400 patients had never been clear since the onset of their disease. Of 260 of his patients treated with a course of ultraviolet light and tar baths, the majority were free for only 6 months, and only three were clear for 5 or more years. Janula & Novotny (1965) found only 13% of their patients had remissions lasting more than 1 year.

Compared with other treatments in a dermatology department, the Ingram technique for psoriasis is time-consuming for patient and staff. If the overall results are as bleak as some of these series suggest, it is debateable whether the service is worth providing. We had the impression that the relapse rate in our treated patients was lower than the above papers imply, but we had no figures to support this view. This investigation was conducted in order to assess the response to treatment in our patients, and the factors influencing the duration of remission from this disease.

METHOD

For this purpose an attempt was made to follow up all those patients first referred to the South Teesside Department of Dermatology in 1966, for treatment of psoriasis. These amounted to 142 patients. In 1973, a letter was sent to each of these, and to their general practitioner, explaining our purpose.

Eventually, ninety-three patients were traced, three of whom had died. This left ninety patients who were interviewed and examined for inclusion in the survey.

The treatments employed in this department are as follows:

(1) *Treatment at home*

This was used for patients with mild psoriasis, or those who were unable to accept more intensive regimens because of other commitments. It entailed regular follow-up at out-patient clinics and the prescription of a variety of topical preparations for the patient to use at home. The duration of treatment in these patients was calculated from the period of out-patient attendance and the subsequent time during which prescribed preparations were used, as far as the patient remembered.

(2) *Out-patient treatment*

This was the Ingram method, where the patient attended the out-patient department daily, Monday to Friday, for a tar bath, graduated exposures to ultraviolet light, and the application of 0·5% Dithranol in Lassar's paste; where indicated a tar and salicylic acid ointment or brief periods with betamethasone valerate cream under polythene occlusion were used also. The duration of treatment was calculated from the number of days they attended, i.e. 5 per week.

(3) *In-patient treatment*

This was similar to that employed above, but the patients received a tar bath and ultraviolet light on 6 days per week, and the topical preparations were applied by the nursing staff each day. The duration of treatment was calculated from the number of days, i.e. 7 per week.

Calculation of duration of remission

For this we had to rely on the patients' memory. This was of necessity imprecise, but almost all patients were able to classify this period into the following time intervals: less than 1 week, 1 week–1 month, 1 month–6 months, 6 months–1 year, 1 year–2 years, and more than 2 years. For any patient receiving more than one course of treatment, the duration of remission was remarkably constant, and before classifying this into one of the above groups, we calculated the mean for each individual.

RESULTS

General parameters

The ninety patients in this survey were all Caucasians, eighty-two (92%) being born in the North-East of England. Twenty-eight (30%) were male, and sixty-two (70%) were female. The mean age at the time of the survey was 40·26 years. The mean age of onset of psoriasis was 24·58 years, 50% of patients developing the condition before the age of 20 years. The mean duration of the condition when first seen was 8·4 years, twenty-five patients (27%) being referred within the first 6 months of the onset of their disease. Thirty-seven patients (41%) gave a positive family history. Two-thirds of these knew of only one relative affected. Exactly half of them had one parent affected, and a third, one sibling.

Treatment. (1) *Previous treatment*

Fourteen patients (16%) had previously been referred to a hospital department. Fifty-three patients (58%) had been treated by their general practitioners. Thirty-three (64%) of these had had some form of topical corticosteroid preparation. The rest had had a tar ointment.

(2) *Treatment on referral to this department*

Of the ninety patients seen in this survey, twenty-eight (29%) were treated at home, fifty-eight (64%) were treated on a daily out-patient basis, and sixteen (17%) were admitted for in-patient treatment.

(3) *Treatment at home*

(*a*) *Duration.* The mean duration of this form of treatment was 22·6 months, the median being 4 months. We agreed with MacLennan & Hellier (1961) that those patients who require an excessively prolonged treatment have a disproportionate effect on the mean, and therefore the median provides a better indication of the effectiveness of treatment.

(*b*) *Results.* Only twelve patients (44·5%) were cleared or much improved after this period; the rest were unchanged by therapy.

(*c*) *Duration of remission.* Of the twelve patients successfully treated at home, eight (66·6%) relapsed within 6 months, and only two maintained their improvement for more than 2 years.

(4) *Daily out-patient treatment*

(*a*) *Duration.* The mean duration was 39 days, the median being 30 days. This is, therefore, considerably shorter than the time taken to treat patients at home.

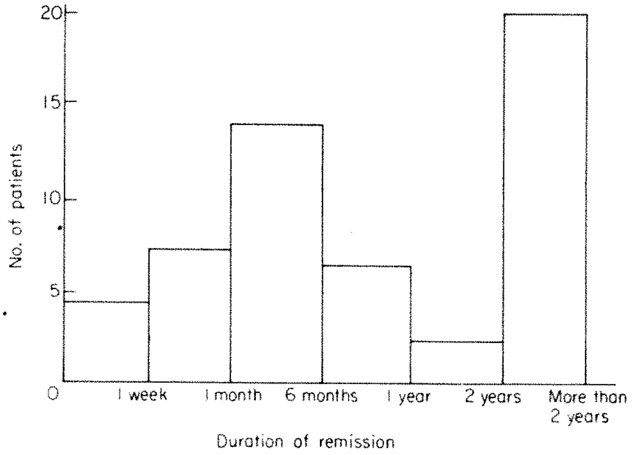

FIGURE 1. Average duration of remission in fifty-three patients who received daily out-patient treatment.

(*b*) *Results.* Thirty-four patients (58%) were clear on discharge, twenty-one (36·2%) were considerably improved, having only a few residual lesions, and only three patients were unchanged by therapy. These, together with another two patients (whose commitments changed so that regular out-patient attendance was no longer possible), were admitted for treatment with a successful outcome.

(*c*) *Duration of remission.* Twenty-five (50%) of the fifty-three patients treated successfully entirely as out-patients relapsed within the first 6 months of discharge. Twenty patients (37%) were clear for more than 2 years, and seven (13%) of the total number had had no relapse in the 7 years of the survey (Fig. 1). Of this last group of seven patients, three had been treated for guttate psoriasis and four for chronic discoid psoriasis.

We looked at the two groups more closely to find a reason for such a difference in the duration of remission. The results are shown in Table 1. From this it seems that the only apparently significant difference between the two groups was the type of psoriasis for which they were treated. Of those

TABLE 1. Factors influencing the duration of remission

	Duration of remission		
	Less than 6 months	Greater than 2 years	Statistical significance
No. of patients	25	20	
Mean age at onset	21·5 years	21·85 years	None
Mean age when treated	30·72 years	27·75 years	None
Incidence of a positive family history	11 patients (44%)	8 patients (40%)	None
Sex	16 females (64%)	15 females (75%)	None
History of topical steroid therapy before out-patient treatment	10 patients (40%)	5 patients (25%)	None
No. of patients improved but not cleared by out-patient treatment	12 patients (48%)	9 patients (45%)	None
No. of patients with guttate psoriasis	0	6 patients	Difference significant

twenty patients who remained clear for more than 2 years, six had had guttate psoriasis, whereas all the other patients in the two groups had chronic discoid psoriasis. In the two groups there was no significant difference between the mean age of onset, age when treated, incidence of a positive family history, sex, history of topical steroid therapy before out-patient treatment, or number of patients improved but not completely cleared by out-patient therapy.

(5) *In-patient treatment*

(*a*) *Duration.* The mean duration was 14·8 days, and the median was 14 days.

(*b*) *Results.* Twelve patients were clear on discharge (80%), the remainder being considerably improved.

(*c*) *Reason for admission.* One-third of patients were admitted because of failure of out-patient treatment, and the remaining two-thirds for failure of home treatment and because of social problems.

(*d*) *Duration of remission.* Thirteen patients (86·5%) relapsed within the first 6 months of discharge, and only two (13·5%) were clear for longer than 2 years. However, we felt that little significance could be attached to these results when comparing them with the outcome of out-patient treatment, because one-third had been admitted for failure of this form of therapy.

(6) *Number of patients returning for further courses of treatment in the 7-year period*

Of the sixty-eight patients who had intensive in-patient or out-patient treatment, thirty-seven (54%) returned for further courses of treatment when their psoriasis relapsed, two patients returning for as many as eight or nine courses.

(7) *Number of patients currently using treatment*

Fifty-three patients (59%) were still using some form of treatment. Of these, twenty-nine were using topical steroids, thirteen tar preparations, and the rest were using ultraviolet light from their own lamps.

DISCUSSION

The epidemiological data collected from our survey were comparable to other much larger surveys on these subjects (Lane & Crawford, 1937; Farber, Bright & Lexie Nall, 1968; Braun-Falco, Burg & Farber, 1972). This suggests that our sample of psoriatic patients was representative of the psoriatic population as a whole. Our results show that if patients attend for out-patient treatment, they not only have a good chance of their psoriasis being cleared, but are likely to stay clear for longer periods than if they are treated at home or in hospital. This is despite the fact that those treated at home were the milder psoriatics. Those treated in hospital tended to be more severely involved; one-third were admitted for failure of out-patient treatment, and therefore the figures are not comparable. It seemed, therefore, that despite the time-consuming nature of intensive out-patient treatment, and the need for trained staff, this was a service worth providing.

For the individual patient, the only significant indicator of the outcome was the type of psoriasis at presentation. Here we are in agreement with Rook, Wilkinson & Ebling (1972), that those patients presenting with guttate psoriasis have a better prognosis than those presenting with chronic discoid psoriasis. In contrast to other series, we were unable to find that a positive family history, an early onset of the disease (Church, 1958), the duration of the condition before treatment was instituted (Ingram, 1954; Romanus, 1945) or the termination of treatment when some lesions were still present (Ingram, 1954), had an adverse effect on the course of the disease.

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Contact dermatitis from plants of the caper family, Capparidaceae

EFFECTS ON THE SKIN OF SOME PLANTS WHICH YIELD *ISO*THIOCYANATES

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SUMMARY

Plants of the caper family (Capparidaceae), which is the tropical relative of the mustard family (Cruciferae), yield 'mustard oil' (*iso*thiocyanate). A review has been made of reported effects on the skin and mucous membranes of some of these plants, of their geographical distribution, and of some opportunities for human contact with them. Plants which yield *iso*thiocyanates can produce irritant dermatitis and some probably can produce allergic contact dermatitis. The Capparidaceae family provides an example of the necessity of recording botanical names rather than common names for plant species, when reporting contact dermatitis from plants.

Capers, which are the pickled flower-buds of *Capparis* species, have been used for culinary purposes to impart a pungent taste to sauces since at least the fifteenth century. Commercial capers were derived from *C. spinaris*, *C. aphylla* and *C. mariana* (Burkill, 1935). The mediterranean *C. spinosa* L. is the species most often marketed at present. The young bud of *Boscia albitrunca* makes an excellent substitute for the caper, and *B. foetida* contains a similarly pungent principle (Watt & Breyer-Brandwijk, 1962). Not everyone has been impressed with this delicacy, for Dioscorides, in the first century A.D., wrote in his Materia Medica: 'Kapparis. [*Capparis spinosa.*] But ye Caper . . . from the Red Sea, and Arabia is extreame sharpe, raysing pustules in the mouth, and eating up ye gummes to the bare bone, wherefore it is unprofitable to be eaten'. There follows a review of subsequently recorded untoward effects of plants of this family.

Clinical reports of untoward cutaneous effects from plants of the family Capparidaceae

(*1*) *Genus* Capparis. Many species are prickly bushes, with a sharp thorn at the base of each leaf. The West African colloquial name 'Wait-a-bit' for *C. erythrocarpa* is probably generally used there for various prickly climbing plants (Dalziel, 1937). The English words for *C. micracantha*, Caper Thorn Melada, are self-explanatory; the Malay word 'Melada' refers to the seeds, spicy and 'peppery' to the taste (Corner, 1952). The flower-buds are used locally as capers in Malaysia. The root of *C. tomentosa* is used as a counter-irritant in southern Africa (Watt & Breyer-Brandwijk, 1962), and the top shoots and young leaves of *C. decidua* (= *C. aphylla*) contain a rubefacient and vesicant principle used in indigenous Indian medicine (Behl *et al.*, 1966).

(2) *Genus* Cleome. Both the seeds and the leaves of *C. icosandra* (= *C. viscosa*) are rubefacient and vesicant (Behl *et al.*, 1966), and *C. viscosa*, named Wild Mustard, is used in West Africa as a counter-irritant (Dalziel, 1937). *C. felina*, found in the Indian States of Maharashtra and Madras, has the same irritant and vesicant properties as has *C. icosandra* (Behl *et al.*, 1966). A gardener developed dermatitis of the hands from working with *C. spinosa*, named Cleopatra's Needle, and patch tests produced an irritant reaction (Szegö & Maácz, 1968). This species is widely distributed in Europe as a half-hardy annual.

(3) *Genus* Courbonia. *C. subcordata* is used as a rubefacient and counter-irritant in East Africa (Verdcourt & Trump, 1969).

(4) *Genus* Crataeva. The root, bark and young shoots of *C. nurvala* are used as rubefacients in India (Behl *et al.*, 1966), and Burkill (1935), from Malaysia, reported that the leaves of this plant were rubefacient and vesicant. The roots of *C. tapia** contain an irritant and vesicant principle, and the wood, when first cut, has a 'garlic-like' odour, and the sap is acrid and caustic and can produce dermatitis (Blohm, 1962; Allen, 1943). The leaf of *C. religiosa* (which is a sacred plant in India) is applied externally as a rubefacient in southern Africa (Watt & Breyer-Brandwijk, 1962); the Yorubas of Africa and the people of the Congo applied the leaf of this plant to the skin as a counter-irritant (Irvine, 1961). *C. lophosperma* and *C. roxburghii* enter into the composition of curries (Corner, 1952).

(5) *Genus* Gynandropsis. The bitter leaves of *G. gynandra* are astringent, with a flavour like that of mustard. The seed has properties similar to those of mustard seed and is used in the same way (Burkhill, 1935; Willis, 1966). The seeds and bruised leaves of this plant and of *C. spinosa* have the same rubefacient and vesicant properties as *Cleome spinosa*, have the same vernacular names in India, and have a general botanical resemblance; according to Behl *et al.* (1966), the plants may in fact be found growing together. The bruised leaf of *G. gynandra* is applied to the skin as a counter-irritant, and the plant yields an acrid 'mustard' or 'garlic'-like oil (Watt & Breyer-Brandwijk, 1962). The seeds and leaves of *G. pentaphylla* are irritant and yield a similar oil. In southern Africa and west Africa the pounded leaf of this plant is applied as a counter-irritant (Watt & Breyer-Brandwijk, 1962; Dalziel, 1937) and in India, the bruised leaf is applied as a rubefacient and vesicant (Chopra, 1933).

The above information is summarized in Table 1.

Biochemical information on the chemical compounds present in these plants

All the species of the caper family, which have so far been investigated, contain thioglucosides of 'mustard oil' type and may therefore be expected to be irritant to the skin (Kjaer, 1960). The occurrence of *iso*thiocyanates or their parent glucosides in plants of the caper family and also in the mustard family (about 165 species), and in a few species of some other plant families, is listed in Tables 2 and 3. Since seeds are available at all times of the year, they have been principally used for such biochemical investigations so far; the green parts of the principal commercial caper species (*C. spinosa*) and of many cruciferous plants have also been found to contain these compounds.

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* It is necessary to use botanical names when describing plants because common, colloquial or vernacular names can be diverse and misleading; for example the following vernacular names have been recorded for *Crateva tapia:* Panama, 'Palo de Guaco'; Venezuela, 'Toco'; British Honduras, 'Waika Bead' or 'Yuy'; Costa Rica, 'Muñeco'; Honduras, 'Cachimbo'; Mexico, 'Cascaron' (Tabasco), 'Kolokmax' or 'Xkolocmax' (Yucatan, Maya), 'Tres Marias' (Yucatan, Spanish) or 'Zapotillo Amarillo' (Colima); Nicaragua, 'Manzana de Playa'; Salvador, 'Anonillo' or 'Granadillo Macho'. Moreover, botanical taxonomy is not static. It is desirable to use botanical names when recording phytochemical information if results of biochemical investigations are to be reproducible by later workers. Thus *Crataeva tapia* Linnaeus (1773) has also received the botanical names (*Crateava gynandra* L. (1762); *Cleome arborea* Schrader (1821); *Crataeva tapioides* De Candolle (1824); *Crataeva acuminata* De Candolle (1824); *Crataeva radiatiflora* De Candolle (1824); *Crataeva benthamii* Eichler (1865) as listed by Blohm (1962). The genus *Crataeva* L. = *Crateva* L. (Willis, 1966); Willis noted that the sign = covers a wide range of meanings but rarely signifies 'is equivalent to'. It means usually no more than that the type/species of the generic name preceding it, is regarded as being congeneric with the type/species of the generic name following it. In this instance Willis (1966) gives the description of the genus under *Crateva* L., which would indicate that he considers this spelling preferable.

The glucoside, glucocapparin, is present in some plants of this family and such glucosides are characterized by the ability to undergo hydrolysis by the enzyme, myrosinase, to yield hydrogen, sulphate, D-glucose and *iso*thiocyanate.

TABLE I. Principal reported effects of plants of the caper family upon the skin and mucous membrane

Genus and species	Principal reported effects upon the skin
Capparis L. 250 species.	Warm areas. Thorn-bearing climbing plants (Willis, 1966)
C. decidua Edgew.	Vesicant (Behl *et al.*, 1966)
(= *C. aphylla* Roth.)	
C. micracantha [sic]	Sharp taste (Corner, 1952)
C. spinosa L.	Irritant (to mucous membrane of mouth) (Dioscorides)
C. tomentosa Lam.	Irritant (Watt & Breyer-Brandwijk, 1962)
Cleome L. DC 150 species.	Tropical and subtropical areas (Willis, 1966)
(*Cleome* L. *sensu stricto* (type *C. gyandra* L.) = *Gynandropsis* DC)	
C. felina L.f.	Irritant (Behl *et al.*, 1966)
C. icosandra L.	Vesicant (Behl *et al.*, 1966)
(*C. viscosa* L.)	
C. spinosa [sic]	Irritant (Szegö & Maácz, 1968)
C. viscosa L.	Irritant (Dalziel, 1937)
Courbonia Brongn. 100 species.	Tropical and southern Africa to India (Willis, 1966)
(= *Maerua* Forsk.)	
C. subcordata Gilg	Rubefacient (Verdcourt & Trump, 1969)
(= *C. tubulosa* Gilg and Benedict)	
Crateva L. 9 species.	Tropical areas excluding Australia and New Caledonia (Willis, 1966)
(= *Crataeva* L.)	
C. nurvala Buch.-Ham	Vesicant (Behl *et al.*, 1966; Burkill, 1935)
C. religiosa Forst.	Rubefacient (Irvine, 1961; Watt & Breyer-Brandwijk, 1962)
(= *C. adansonii* Oliv.)	
C. tapia L.	Vesicant (Allen, 1943; Blohm, 1962)
Gynandropsis DC (~*Cleome* L.)	One species. Tropical and subtropical area (Willis, 1966)
G. gynandra Bricq.	Irritant (Behl *et al.*, 1966; Watt & Breyer-Brandwijk, 1962)
G. pentaphylla DC	Vesicant (Watt & Breyer-Brandwijk, 1962; Dalziel, 1937; Chopra, 1933)

In this instance a simple 'mustard type' *iso*thiocyanate, CH_3NCS, is the product of the glucoside.

$$\begin{array}{c}\text{Glucocapparin}\\ \text{(Thioglucoside)}\end{array} \xrightarrow[\text{(Enzyme)}]{\text{Myrosinase}} \begin{array}{c}CH_3NCS\\ \text{(}\textit{Iso}\text{thiocyanate.}\\ \text{'Mustard' oil)}\end{array}$$

The compound, CH_3NCS, is released by the same form of hydrolysis as occurs when siningrin, the glucoside of the seeds of mustard (*Brassica nigra* Koch), undergoes fission under the action of the enzyme, myrosinase, to yield allyl*iso*thiocyanate.

$$\underset{\text{(Thioglucoside)}}{\text{Sinigrin}} \xrightarrow[\text{(Enzyme)}]{\text{Myrosinase}} \underset{\text{(}\mathit{Iso}\text{thiocyanate. Mustard oil)}}{CH_2=CHCH_2NCS}$$

TABLE 2. The occurrence of *iso*thiocyanates or their parent glucosides in the caper family (Kjaer, 1960)

Family	Species	Source	Compound	Derived
Capparidaceae, caper family (including Cleomaceae)	*Capparis spinosa* L.	Green parts of plant Seeds	Glucocapparin (also known in crystalline form and as crystalline tetra-acetate)	*Iso*thiocyanate (CH_3NCS)
	Cleome arabica L.	Seeds*	,,	,,
	C. arborea Bss.	Seeds*	,,	,,
	C. gigantea L.	Seeds*	,,	,,
	C. graveolens Rafin	Seeds*	,,	,,
	C. monophylla L.	Seeds*	,,	,,
	C. speciosissima Deppe	Seeds*	,,	,,
	C. spinosa Jacq.	Seeds*	,,	,,
	C. trachysperma (Torr. and Gray) Pax and K. Hoffm.	Seeds*	,,	,,
	C. visocsa L.	Seeds*	,,	,,
	Gynandropsis gynandra (L.) Briq.	Seeds	,,	,,
	G. pentaphylla DC	Seeds†		

* In addition to glucocapparin, an unidentified glucoside is present (Kjaer, 1960).

† The glucoside is unidentified (Kjaer, 1960).

TABLE 3. The occurrence of *iso*thiocyanates or their parent glucosides in some other plant families (Kjaer, 1960)

Family	Species	Source	Derived
Caricaceae	*Carica papaya* L.	Seeds	*Iso*thiocyanate (R-NCS) (R = as listed by Kjaer, 1960)
Cruciferae	About 165 species	For separate review	
Euphorbiaceae	Two species	For separate review	
Limnanthaceae	*Limnanthes douglasii* R. Br.	Seeds	
Moringaceae	*Moringa pterygosperma* Gaertn.	Root	
Phytolaccaceae	*Codonocarpus cotinifolius* (Desf.)	Green parts	
Plantaginaceae	*Plantago major* L. (?)*	Green parts	
Resedaceae	*Reseda alba* L.	Root	
	Reseda lutea L.	Green parts, root	
	Reseda luteola L.	Root, green parts, seeds	
	Reseda odorata L.	Root, seeds	
Salvadoraceae	*Salvadora oleoides* Den.	Seeds	
Tropaeolaceae	*Tropaeolum majus* L.	Green parts, seeds	
	Tropaeolum peregrinum (canariense) [*sic*]	Seeds	

* Uncertain botanical identification of the species examined (Kjaer, 1960).

The root of horse-radish, *Armoracia lapathifolia* Gilib., serves equally well. When applied to the tongue, all mustard oils cause a sharp and burning sensation. Like most synthetic mustard oils, those of natural origin show vesicant properties (Kjaer, 1960).

These compounds have received the name mustard-oils (some are solid at certain temperatures or volatile) because they are characteristic of and were indeed first isolated from mustard plants, family Cruciferae. The name, therefore, in a sense indicates their chemical properties rather than their botanical origin; they are best designated by the name, *iso*thiocyanate, because they have been found as derivatives of, so far, about a dozen botanical families (Tables 2 and 3).

TABLE 4. Reported effects on the skin of some plants which are listed in Tables 2 and 3 as containing thioglucosides

Botanical name	Common name	Effects
Carica papaya L.	Yields papaya fruit	The seeds contain thioglucosides, and myrosinase is present in the fruit pulp. Contact with the juice of the edible fruit, papaya, can cause dermatitis and conjunctivitis (Watt & Breyer-Brandwijk, 1962; Souder, 1963; Hardin & Arena, 1969). The juice contains the proteolytic enzyme, papain.
Moringa spp.	Horse-radish tree	The root if applied to the skin acts as a mustard plaster and was used as such (Burkill, 1935). The crushed leaves are vesicant (Allen, 1943). In India, the thickened roots of young plants are used as a substitute for horse-radish (Dalziel, 1937). The root and bark are used in West Africa for counter-irritant purposes (Dalziel, 1937; Irvine, 1961).
Phytolacca spp.	Pokeweed	The green parts and roots are irritant to the skin and conjunctivae (White, 1887; Chopra *et al.*, 1958).
Reseda odorata L.	Mignonette	Rubefacient (Burkill, 1935).
Salvadora spp.	Mustard tree	The plant has been thought by some (probably erroneously) to be the mustard of the Bible; its leaves taste like mustard (Willis, 1966). The bruised root-bark is vesicant (Behl *et al.*, 1966). A paste of the powdered root, used like a mustard plaster, has a vesicant effect.
Tropaeolum majus L.	'Nasturtium'	Rubefacient (Burkill, 1935). Can produce dermatitis of the hands in gardeners (White, 1887; Pammel, 1911).

Botany and geographic distribution of plants which yield the iso*thiocyanate*, CH_3NCS

The family Capparidaceae Juss. (*sensu stricto*)* comprises dicotyledonous plants, of thirty genera, 650 species occurring in tropical and warm temperate geographic areas (Willis, 1966). They are characteristically small trees or shrubs, often thorny. In simplest terms, we are dealing with the caper family, including the *Gynandropsis* = *Cleome* relatives. The caper family (Capparidaceae) is regarded as the tropical relative of the mustard family (Cruciferae), the latter being distributed largely in temperate regions (Good, 1964).

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* In the strict sense, the family has been reduced by exclusion of certain genera, notably *Cleome* L. DC and *Gynandropsis* DC, which have been afforded a separate family Cleomaceae (Pax) Airy Shaw under rules of botanical nomenclature. *Sensu lato*, the family Capparidaceae has included the genera *Cleome* and *Gyandropsis* (Hutchinson, 1959). Another taxonomic complexity which should be mentioned is that *Gynandropsis* DC is alternatively named *Cleome* L. DC, and that *Busbeckea* (*B. arborea*, the caper-tree of New South Wales) = *Capparis* L. These matters of nomenclature are recorded by Willis (1966) and will not be elaborated further here.

Search for clinical reports of dermatitis from related chemical compounds

In Table 4 are listed some reported effects on the skin of the plants which are listed in Table 3 as containing thioglucosides (glucosinolates).

Conjunctivitis and dermatitis from the papaya fruit, *Carica*, may result from the proteolytic enzyme, papain, which is present in the juice, rather than from the thioglucosides, which are present in the seeds. The effects of cruciferous plants upon the skin may well result from the thioglucosides which are present in many species. The dermatitis caused by contact with the milky white latex of euphorbiaceous plants is more likely to be due to compounds other than thioglucosides. These families will be reviewed separately. The notes on *Moringa*, *Phytolacca*, *Reseda*, *Salvadora* and *Tropaeolum* seem self-explanatory.

TABLE 5. Opportunity for human exposure to some plants of the caper family

Botanical name	Common name	Opportunity for exposure
Capparis spinosa	Spider-flower	Cultivated and naturalized to waste grounds or alluvium, Florida to Texas, north to Massachussets, New York, Ohio, Indiana, etc. (Gray's Manual, 1970). Straggling spiny shrub 3 feet or more high (Bailey's Manual, 1971). Source of the caper used for food.
Cleome spinosa (*C. pungens*, *C. gigantea*)	Giant spider-flower	Strong scented annual 3–4 feet high. Flowers numerous, rose-purple or white. Adventive, New York to Louisiana. Grown in background as summer hedge, against walls or fences, in large containers or naturalized in fringe areas of the garden. Pink and white flowered varieties. Flowers and dry capsules are useful in arrangements.
	Spiny spider flower	In recent years has become a very popular garden annual even though it is strong scented (Wyman, 1971).
Cleome serrulata	Stinking clover or bee	Spider flower (Wyman, 1971) and *C. lutea* (Bailey's Manual, 1971) are also grown as garden annuals.
Gynandropsis		Material grown under this name may sometimes belong to *Cleome spinosa* (Bailey's Manual, 1971).

Survey of opportunity for human exposure to some plants which yield thioglucosides

As will be seen from Table 5, there is opportunity for exposure to some of these plants for the home-gardener, notably to the spider-flowers, one of which, *Cleome spinosa*, has become a popular garden annual (Wyman, 1971).

About 165 species of Cruciferae have so far been found to yield mustard oils (Kjaer, 1960). The familiar condiment, table mustard (*Brassica*), and the well-known effects of a mustard plaster upon the skin are characteristic examples of the pungency and irritancy of this group of compounds. The subject will be reviewed elsewhere (Mitchell & Rook, 1974), but it may be briefly noted here that a mustard oil has been clearly demonstrated to produce allergic contact dermatitis in guinea-pigs (Landsteiner & DiSomma, 1938), and that there is clinical evidence to support such a possibility in man (Gaul, 1964). Alleged photodermatitis from these plants may possibly be no more than a combination of sunburn and of irritant dermatitis described by Szegö & Maácz (1968).

DISCUSSION

In the past few decades, there has been increasing interest in the chemotaxonomy of plants and, in the botanical and phytochemical literature, there is a rich harvest of information concerning plants and chemical compounds of dermatological interest. From this literature it is possible to draw up lists of 'potentially irritant' and of 'potentially allergenic' plants.

In the caper family, the growth of knowledge of potential dermatological interest has indeed been explosive. Mustard has figured as a condiment and rubefacient in the Herbals for many centuries. In 1831 a mustard-oil glucoside was first isolated, a second was isolated in 1913; up to 1952 eight were recorded, and by 1960 about thirty (Kjaer, 1960); in 1963 Ettlinger & Kjaer reviewed some fifty thioglucosides. These authors and Underhill & Wetter (1966) discussed the biosynthesis of these compounds.

From this review, the harvest of 'potentially irritant' plants for the home-gardener in temperate climates is meagre, which is not surprising when one considers that the caper family is largely tropical; but when this method of review is applied to the formidable number of characteristically temperate cruciferous plants which yield mustard oils, it is apparent that a substantial number of garden and vegetable plants are 'potentially irritant' if not 'potentially sensitizing'.

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Induction of delayed hypersensitivity to nitrogen mustard in the guinea-pig

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SUMMARY

The acquisition of allergic contact dermatitis limits the therapeutic usefulness of topical nitrogen mustard in man. A reproducible experimental model for this syndrome has been provided in the guinea-pig. Guinea-pigs were sensitized to nitrogen mustard (HN_2) by means of the repeated epicutaneous administration of sensitizer to a skin site, and the injection into that site of complete Freund's adjuvant. Application of dimethyl sulphoxide before HN_2 to the sensitization or challenge site facilitated the sensitization and challenge procedures. Positive challenge could be elicited by the intradermal injection of a non-toxic amount of HN_2. The methodology extends to the study of a variety of other sensitizers and potential sensitizers.

Nitrogen mustard (mechlorethamine, $CH_3\text{-}N(CH_2CH_2Cl)_2$) is an alkylating agent, widely used in cancer chemotherapy. It is unstable at alkaline or neutral pH, but relatively stable in acid solutions. It forms covalent reaction products with a wide variety of biological materials, including nucleic acids and proteins. It is likely that the major biological effects produced by this drug result from its forming irreversible cross linkages, since, generally, monovalent alkylating agents are relatively inactive (Wheeler & Alexander, 1969).

Topical nitrogen mustard has been used with considerable success in the treatment of mycosis fungoides (Haserick, Richardson & Grant, 1959; Madison & Haserick, 1962; Van Scott & Wruters, 1970). Repeated application to lesions of mycosis fungoides, particularly to those in the erythematous or plaque stage, may cause them to resolve, sometimes for a long period of time. Topical nitrogen mustard has also been reported to induce healing in stable plaques of psoriasis (Epstein & Ugel, 1970).

The principal toxic side effects of parenterally administered nitrogen mustard, that limit the amount of drug that can be given, are bone marrow depression and gastro-intestinal ulceration. Significant absorption of nitrogen mustard does not occur when it is applied to the skin. However, the induction of allergic contact dermatitis is a frequent complication of therapy with topical nitrogen mustard. For instance, of the patients of Epstein & Ugel (1970) who were treated with topical nitrogen mustard, the drug had to be discontinued in half because they became sensitized to the medication. Further, beyond the usual hazards and discomforts of allergic contact dermatitis, in psoriasis there is the real possibility of a Koebner phenomenon developing at sites of contact dermatitis. A recent attempt to induce specific immune tolerance to nitrogen mustard in patients by giving the drug intravenously prior to topical therapy was reported as unsuccessful (Baer, Michaelides & Prestia, 1972).

An animal system in which the acquistition and expression of allergic contact sensitivity to nitrogen mustard could be studied, would seem desirable. We now report experiments in which allergic contact dermatitis to nitrogen mustard has been induced in guinea-pigs with some regularity.

MATERIALS AND METHODS

The animals were closed-colony, randomly bred, Hartley strain, or Family 13 derived, guinea-pigs. In different experiments, either males or non-pregnant females weighing between 300 and 500 g were used. The animals were fed fresh Purina Guinea-pig Chow supplemented with lettuce thrice weekly; water *ad libitum* was provided. The guinea-pig colony was clinically free of streptococcal C cervical adenitis and the animals were considered generally healthy as judged by consistent weight gain and negligible unintentional losses.

The guinea-pigs were sensitized by a variant of the 'split adjuvant' technique, that is by the separate successive administration of allergen (Mustargen®, Merck) and complete Freund's adjuvant to the skin (5 mg heat-killed tubercle bacilli of the H37Ra strain in 10 ml of a solution consisting of 8·5 ml mineral oil and 1·5 ml Arlacel A, Difco) (Maguire & Chase, 1967). Challenges were made to new, freshly clipped sites on the back, the appearance of the induced reactions being observed and recorded daily for several days. Later, the intensity of the inflammation (erythema, induration) was scored: 0, ±, +, + +, . . . + + + + + + (Chase, 1954). Readings less than '±' were described as 'strong trace', 'trace', 'faint trace', or 'very faint trace' to differentiate these reactions from those in which the challenge site appeared normal, i.e. '0'. Intradermal reactions were evaluated for size, with calipers, and for thickness, by palpation. Unsensitized toxicity control guinea-pigs were always tested in parallel. Skin biopsis were taken under light ether anaesthesia and fixed in neutral buffered formalin. The biopsy specimens were processed to sections by routine histopathological techniques.

RESULTS

In a typical experiment, we prepared a sensitization site on the right dorsal flank of eight male Family 13 derived guinea-pigs. The skin was clipped with animal clippers and then close-shaved with a safety razor. Then 0·1 ml of a freshly prepared 0·1% solution of nitrogen mustard in distilled water was applied to a 1 cm square filter paper (Whatman No. 3) and this in turn put on the shaved site. The filter paper was covered with occlusive tape (Blenderm®, Minnesota Mining Company) and this fixed in place with a stretch adhesive (Elastoplast®, Duke Laboratories) and regular adhesive tape. The allergen was reapplied to the filter paper on days 2, 4, and 7, and the dressings removed on day 9. On day 4, complete Freund's adjuvant, 0·1 ml was injected intradermally on either side of the sensitization site (Maguire, 1973). Boosting applications of sensitizer were made on days 21 and 28 under closed dressing. On day 35, animals were tested intradermally with nitrogen mustard 0·001%, (0·1 ml in volume) intradermally. They were further tested on day 45 topically on two sites with 0·01 ml of 1% nitrogen mustard (HN_2); this solution was pipetted and spread over an area measuring about 1 cm diameter; on one site, 0·01 ml of a solution containing nine parts dimethyl sulphoxide (DMSO) and one part water was applied about 10 min prior to challenge with HN_2. The results of these testings are outlined in Table 1.

In another experiment, we sensitized two groups of male Hartley guinea-pigs to HN_2 using the open application to the skin of a 1% (w/v) solution of HN_2 in normal saline. In one of the experimental groups, we pretreated the sensitization site with DMSO prior to the percutaneous administration of the HN_2 solution (Table 2). Thus, DMSO (90%, 0·01 ml) was applied to a single clipped site on the right rear dorsal flank on days 0, 1, 2, 3, 4 and 7. On each of these six days, about 10 min later, HN_2 (1%, 0·01 ml) was pipetted onto the same site (guinea-pigs nos. 278–281) or onto a clipped site in a symmetrical location on the left side (guinea-pigs nos. 282–285). On day 4, complete Freund's adjuvant (0·1 ml) was injected into the HN_2 sensitization site in all eight animals. Testings were made on day 16 with 0·5 ml HN_2 (with and without DMSO pretreatment of the challenge site), and on day 22 by the intradermal route with 0·1 ml of 0·0001% HN_2 in normal saline. The results are summarized in Table 2. Clearly, guinea-pigs could be sensitized to HN_2 by this open technique, and the efficiency of the method was much improved by prior treatment of the sensitization site with DMSO.

TABLE 1. Challenge reactions of guinea-pigs sensitized to nitrogen mustard under occlusive dressing

Guinea-pig no.	Intradermal, T-35	Topical, T-43		
	HN_2	HN_2	DMSO, HN_2	DMSO
193	11	+ + +	+ + + + ±	[*tr*]
194	9	+ +	(+ + + +)	f.tr
195	8	+ ±	(+ + + +)	f.tr
196	<3	[±]	(+ + +)	0
197	5	+ +	+ + + +	0
198	[9]	+ + +	+ + + + +	0
199	9	(±)	+ + + +	f.tr
200	6	+ ±	[+ + + + +]	[tr]
TC	0	---	---	---
TC	0	---	---	---
TC	---	0	±	0
TC	---	0	(±)	0

Eight guinea pigs were sensitized to nitrogen mustard by means of a modified split adjuvant technique using an occlusive dressing. The results of intradermal testing with 0·001% (w/v) HN_2 on day 35, and of epicutaneous testing with 1% HN_2 (to sites untreated and pretreated with 0·01 ml of a 90% DMSO aqueous solution 10 min previously) and with 90% DMSO alone on day 43 are shown. The diameters in millimeters of the intradermal reactions are recorded; the intensity of the inflammatory response evoked by the topical applications have been scored according to standard criteria. For f.tr read faint trace; tr, trace; *tr*, strong trace. Round brackets ( ) indicates maximum reaction at 48 h, square brackets [ ], maximum at 72 h. For TC read unsensitized toxicity control.

Biopsy was made of the challenge reactions at 48 h, in guinea-pigs nos. 279 and 280 (Table 2). In sections of specimens derived from sites tested with nitrogen mustard alone, the epidermis showed spongiosis, extracellular oedema and exocytosis; in the upper dermis there was a round cell infiltrate. The histopathological changes were consistent with allergic contact dermatitis in the guinea-pig (Turk, 1967). The site challenged in succession with DMSO and HN_2 showed moderate to marked oedema of both dermis and epidermis; there were clefts, exocytosis and foci of necrosis in the epidermis, and the infiltrate contained polymorphonuclear leukocytes as well as lymphocytes.

TABLE 2. Challenge reactions in guinea-pigs sensitized to nitrogen mustard with and without pretreatment of the sensitization site with DMSO

Guinea-pig no.	T-16, topical			T-22, intradermal
	HN_2	DMSO, HN_2		HN_2
278	+ + +	+ + + + +		8
279	+ + + ± (biopsy)	+ + + + + (biopsy)		10
280	+ + + + ± (biopsy)	+ + + + ± (biopsy)		9
281	(+ +)	(+ + +)		10
282	0	+ +		0
283	+	+ + + ±		f.tr
284	+	+ +		tr
285	0	+ +		< 3
TC	0	tr ?	TC	0
TC	0	*tr*	TC	< 3
TC	0	0	TC	< 3
TC	0	0	TC	< 3

Guinea-pigs were sensitized by the application of 0·01 ml of HN_2 to the skin utilizing complete Freund's adjuvant. In four of the guinea-pigs, the sensitization site was treated each time with 90% DMSO prior to the application of HN_2; the other four received DMSO to the opposite flank prior to HN_2. The results of topical testing on day 16 with 0·01 ml of 0·5% (w/v) HN_2 (to clipped sites with and without prior preparation with 0·01 ml 90% DMSO), and on day 22 with 0·01 ml of 0·0001% HN_2 in saline (intradermally) are indicated. The scoring system is outlined in Methods. TC: unsensitized toxicity control.

TABLE 3. Challenge tests to nitrogen mustard in recipients of cells from sensitized donors

Guinea-pig no.	Day				
	0	1	2	3	4
364	PE cells		+ ±	±, biopsy	– – –
365	PE cells		+ ±	+ ±, biopsy	– – –
366	Spleen cells		*tr*	tr	0
367	Spleen cells		f.tr.	0	0
368	– – –		prac. 0	0	0
369	– – –		0	0, biopsy	– – –
370	– – –		0	0, biopsy	– – –
371	– – –	HN_2	0	0	0

The results of contact tests with 1% HN_2 to recipients of viable cells from sensitized guinea-pigs, and to toxicity controls (no cells), are shown. The scoring system is given in Methods. Biopsies of the 48 h reactions in nos. 364, 365, 369 and 370 precluded 72 h readings in these animals. For 'PE' read 'peritoneal exudate'.

A limited attempt was made to transfer HN_2 hypersensitivity passively in the guinea-pig. Donor animals were the eight experimental guinea-pigs listed in Table 2. On day 32, they were injected intraperitoneally with 13 ml of medium weight mineral oil. Two days later, they were killed by acute cervical dislocation, and their spleen and peritoneal cells collected in separate pools by the method of Bloom & Chase (1967). 260×10^6 peritoneal exudate cells were injected intraperitoneally into each of two fresh guinea-pigs (nos. 364 and 365). Two further recipients (nos. 366 and 367) were each given 245×10^6 spleen cells, again intraperitoneally. The results of contact tests made with 0·01 ml of 1% HN_2 to clipped sites are shown in Table 3. The recipients of peritoneal cells, but not of spleen cells, showed definite positive reactions. Biopsies of the positive reactions revealed a mild dermatitis with a mononuclear infiltrate compatible with allergic contact dermatitis. Perhaps the use of a larger number of spleen derived WBCs would have resulted in a successful transfer. Peritoneal cells have been shown to be more active than spleen cells in transferring reactions of delayed hypersensitivity (Bloom & Chase, 1967). Waldorf, Haynes & Van Scott (1967) reported the successful transfer of contact sensitivity to nitrogen mustard in humans using about 10^9 blood leukocytes obtained from exquisitely sensitive donors.

DISCUSSION

Clinically, nitrogen mustard is a sensitizer of intermediate strength in man; it is a weaker sensitizer than dinitrochlorobenzene (DNCB) or picryl chloride, but stronger than materials such as formalin or procaine. This ordering appears to hold for the guinea-pig.

Guinea-pigs can be sensitized to an aqueous solution of nitrogen mustard by a closed technique or by an open method. In this context, a general difficulty with aqueous solutions is their poor penetration through the barrier layer of the epidermis. Occlusion facilitates such penetration and this is a major advantage of the closed technique (Magnusson & Kligman, 1970). Dimethylsulphoxide increases the penetration of materials through the skin by altering the physico-chemical constitution of the barrier layer (Stoughton & Fritsch, 1964; Kligman, 1966; Baker, 1968). Almost certainly, this accounts for the regular increase in the intensity of the challenge reactions in sites pretreated with DMSO; in fact, in our experiments, minor primary irritant reactions developed in several toxicity control animals that received in turn DMSO then HN_2.

Pretreatment of the sensitization site with DMSO enhanced the acquisition of allergic contact sensitivity in guinea-pigs so treated. While this can be a useful experimental manipulation, the potential hazard for the worker handling DMSO and allergenic (or toxic) compounds at the same time must be kept in mind. We have previously described a technique for identifying potential contact allergens in the guinea-pig by repeated applications of test material under occlusion to a sensitization site and the injection of complete Freund's adjuvant next to the site. However, prolonged closed dressings are inconvenient and may irritate the skin of the guinea-pig. It is likely that as an alternative many materials (especially liquids) can more easily be investigated for their allergic potential by substituting for occlusive dressing the pretreatment of the sensitization site with DMSO.

ACKNOWLEDGMENTS

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Percutaneous absorption and the surface area of occluded skin

A SCANNING ELECTRON MICROSCOPIC STUDY

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SUMMARY

Scanning electron microscopy of samples of human skin occluded for 72 h, revealed that the hydrated stratum corneum not only swells, but develops multiple folds. Surface area estimations of such stratum corneum, utilizing stereo pairs of the photomicrographs, indicated a 37% increase over the normal, non-occluded horny layer values. It is speculated that the increase in absorptive area contributes to the increased skin permeability following occlusion.

The most widely used method of increasing percutaneous absorption of topically applied therapeutic agents is by the use of occlusive plastic films (Garb, 1960; Hall-Smith, 1962; Scholtz, 1961; Sulzberger & Witten, 1961). With this technique, absorption of some substances can be increased by as much as 100 times, in addition to the formation of a long-lasting reservoir within the stratum corneum (Bettley, 1970). Fritsch & Stoughton (1963) have shown that, in occlusive environments approaching 100% relative humidity, increased penetrability is due almost entirely to the hydration of the stratum corneum.

In the course of studies in which skin was occluded for 72 h and prepared for examination with the scanning electron microscope (SEM), the present authors noted remarkable alterations in the appearance of the horny layer of the occluded skin. The possible influence of such changes on percutaneous absorption seemed obvious, since the area of the absorptive surface was apparently increased. The potential increment was assessed using SEM photomicrographs and mathematical estimation techniques.

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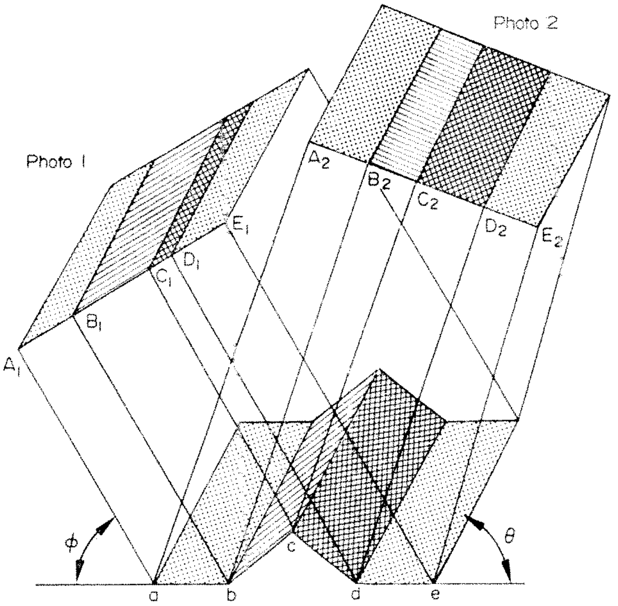

FIGURE 1. Diagrammatic representation of the principle of photogrammetric estimation, where two photographs of the same object taken at different angles are used to calculate measurements of height.

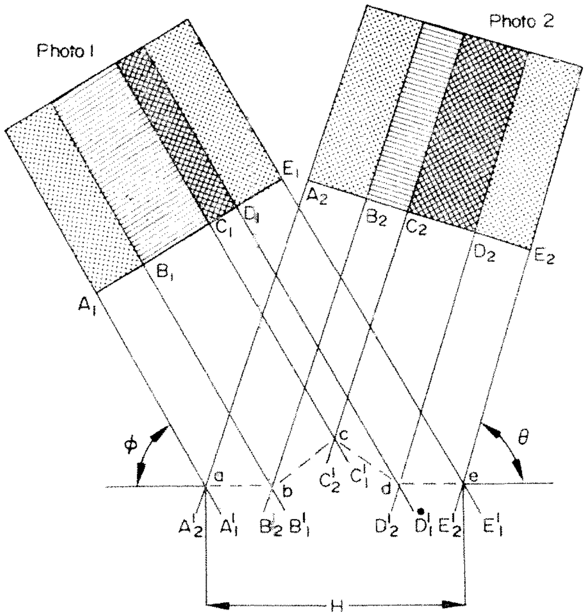

FIGURE 2. Simplified diagram of a planimetric technique where the photographic series is arranged in such a way that the intersections of perpendicular lines through known points completes a topographical profile.

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METHODS

Subjects

Ten healthy male volunteers were included, none of whom had previous skin disease.

Histological preparations

Two 3 mm dermal punch biopsies were taken from each subject; one from a site occluded for 72 h with firmly affixed polyethylene film, and one from an un-occluded site. The specimens were dehydrated in graded alcohols, air dried and fixed to mounting blocks with the horny layer surfaces facing upward. The tissues were coated with gold–palladium in a vacuum evaporator and examined in a Jelco JSM-2 Scanning Electron Microscope. Photographs of the resulting images were taken of the samples at angles of 0°, 5°, 10° and 15°.

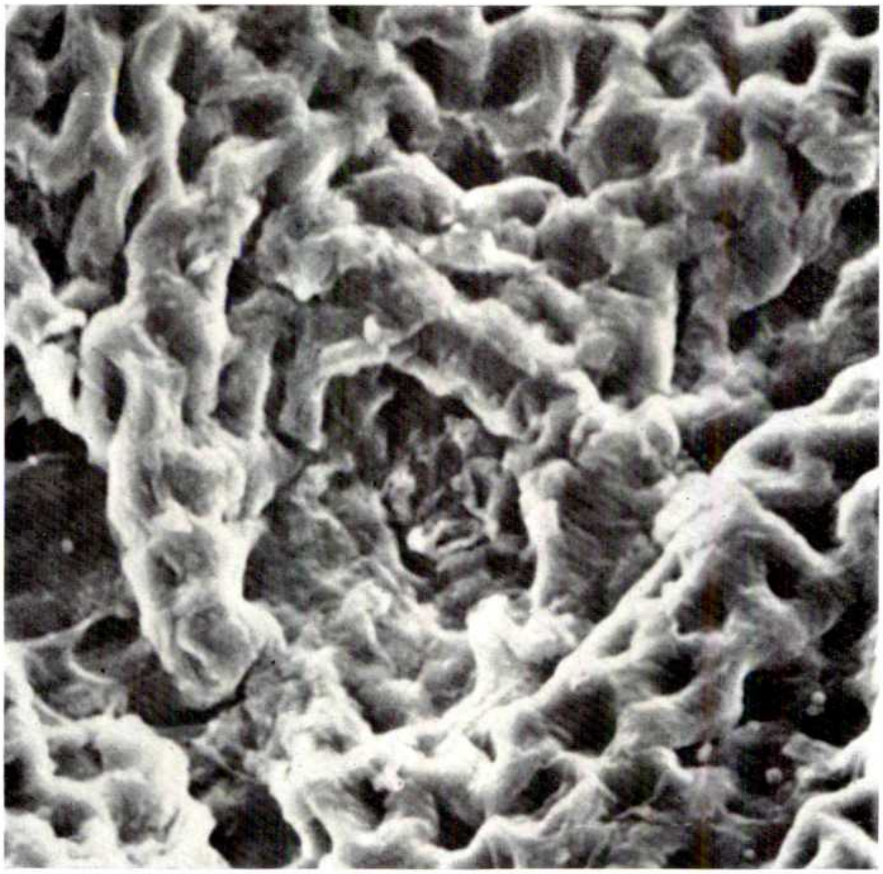

FIGURE 3. Skin occluded for 72 h. The horny layer is swollen and thrown into folds. Individual, desquamating cells are difficult to visualize. The oval structure in the centre of the field is an eccrine sweat pore (× 500).

Estimation of surface area

The basic approach was to use two photographs of the same object taken from different angles (Fig. 1) and geometrically calculate measurements of height from them. When making topographical maps, the photographs are taken from two different locations, usually from an aeroplane (Thompson, 1966). In our case, the camera was fixed and the prepared sample was rotated to obtain different views.

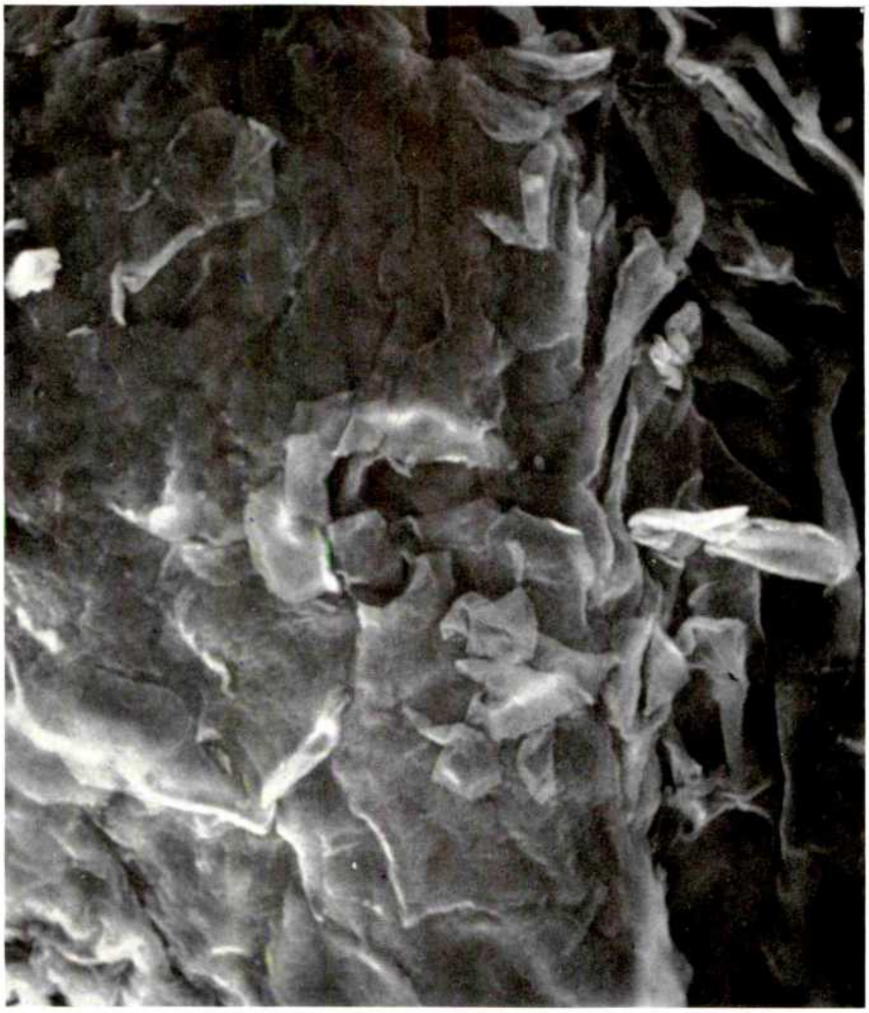

FIGURE 4. Non-occluded skin. The relatively flat surface and shedding of stratum corneum cells is the normal picture. The central oval structure is an eccrine pore (× 500).

Resulting photographs were then used to plot a series of profiles or cross-sections taken through the sample perpendicular to the main plane of the surface. A line was drawn in the same place on each photograph through which a profile was to be made. These are shown as lines A_1–E_1 and A_2–E_2. The photographs were laid out on a drawing board as shown in a simplified profile (Fig. 2), such that angles ϕ and θ are the same as ϕ and θ in Fig. 1. Lines A_1–$A_1{}^1$ to E_1–$E_1{}^1$, and A_2–$A_2{}^1$ to E_2–$E_2{}^1$ were drawn perpendicular to the lines A_1–E_1 and A_2–E_2. The point *a* on the profile is determined by the intersection of lines A_1–$A_1{}^1$ and A_2–$A_2{}^1$ and point *b* is determined by the intersection of lines B_1–$B_1{}^1$ and B_2–$B_2{}^1$, etc. In this way, a series of known points on the profile were located. Using the photographs as a reference, a line was then drawn which connects all the points, thus completing the profile (Thompson, 1966; Robinson, 1969).

In the simplified example shown in Figs. 1 and 2, all profiles taken parallel to a–e would be identical, but with the more complicated surfaces of the stratum corneum samples, this was not the case. Therefore, several different profiles were made. The length of the line forming each profile was measured and divided by the horizontal length (H), giving a unit profile length (L). An average L value for both the un-occluded and occluded samples was calculated. An approximation of the unit surface area was found by squaring the resulting average L values. The ratio of L^2 for occluded stratum corneum to L^2 for un-occluded stratum corneum would thus be an approximation of the ratio of the two areas.

RESULTS

Skin occluded for 72 h becomes swollen and thrown into undulating folds (Fig. 3). This picture is an obvious distortion of the normal, predominantly flat or plate-like, layered texture of the stratum corneum seen in SEM images of un-occluded skin (Fig. 4).

The ratio (L_o) of the average surface area calculated for the occluded specimens compared to an ideal horizontal line (H) was 1·30. For the un-occluded specimens the ratio (L_u) was 1·11. The ratio of these average L values $1.30^2/1{\cdot}11^2$ was found to be 1·37. In other words, according to these calculations, the occluded sites had 37% more surface area than the un-occluded.

DISCUSSION

When the stratum corneum becomes hydrated under an occlusive dressing, the tissue changes from one containing little water (5–15%) to one which may contain as much as 50% water (Blank & Scheuplein, 1964). For the most part, the water is held in spaces between fibre bundles, swelling the interfibrillary structure of the stratum corneum, straightening the polar side-chains, and forming a more or less continuous aqueous pathway through part of it (Yates, 1971). Virtually all of the resulting increased rate of transport of water-soluble substances has been attributed to this internal change in the physical structure of the membrane (Fritsch & Stoughton, 1963; Blank & Scheuplein, 1964; Yates, 1971).

However, even cursory examination of the SEM images (Figs. 3 and 4) reveals that stratum corneum hydrated by polyethylene occlusion has a greater surface area than un-occluded specimens. It is reasonable to assume that the increased surface area contributes to increased cutaneous penetrability which follows occlusion.

It is worth noting that the simple, graded alcohol preparation of skin for SEM preserves the swollen, convoluted morphology of the occluded samples. The end point of preparation, dryness, does not restore their structure to the flat surfaces of non-occluded skin. As has been previously noted, the fixation which attends gradual dehydration of skin apparently minimizes the production of artifacts (Papa & Farber, 1971).

Our mathematical determination of the relative increase in surface area of occluded skin is, at best, a crude estimate. Equipment similar to that used for converting aerial photographs of earth surface terrain into line topographical maps (stereo comparagraph) could be used to obtain more precise information.

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The influence of enzyme detergent on skin reactions to beryllium

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SUMMARY

The incorporation of enzyme in a detergent was not shown to promote sensitization or positive patch test reactions in albino guinea-pigs.

Incrimination of enzyme detergents in the production of contact dermatitis in housewives has received rather little support. This is probably because the tests carried out by Griffith, Weaver & Whitehouse (1969) and by Bolam, Hepworth & Bowerman (1971), although thorough, did not reveal an irritant or sensitizing potential. The case reports (Ducksbury & Dave, 1970; Jensen, 1970) did not suggest an allergic response, and indeed the mechanism by which the eruptions were supposed to arise remained obscure.

It seems, however, that a possible mechanism has not received adequate consideration. It is known that many detergents when brought into contact with the skin increase the permeability of the corneous layer and may thus enhance the effect of an irritant or sensitizer which is applied at the same time. It may be, therefore, that a dermatitis, following and attributed to contact with a detergent may in reality be due not to that substance, but to some other irritant or sensitizer, the action of which has been facilitated by the detergent. Neither Ducksbury & Dave (1970) nor Jensen (1970) mentioned that this possibility was fully proved in their cases.

One may surmise that if the presence of an enzyme influences epidermal permeability, it will have a corresponding effect on the incidence of contact dermatitis induced by simultaneous or subsequent exposure to other substances.

Some measurements of water-permeability of excised human epidermis did indeed show that the increase of permeability brought about by a detergent was enhanced if enzyme were present (Bettley, 1972). This must not of course be taken to indicate that permeability to all other substances would similarly increase, but that this may apply to metallic cations was suggested in that a similar increase in permeability to potassium was found.

The present series of experiments was carried out in order to see, if possible, whether enzyme can in fact be shown to increase the chance of sensitization to other contactants or to promote the development of reactions in those already sensitized.

Three questions were considered:

(1) Does the application of enzyme detergent simultaneously with a sensitizer increase the chances of sensitization?

(2) Does pretreatment of the skin with an enzyme detergent increase the chances of sensitization by a sensitizer subsequently applied?

(3) Does the incorporation of enzyme detergent in the test solution increase the chances of a positive patch-test reaction in an already sensitized animal?

The test subjects were adult female albino Hartley guinea-pigs. The sensitizer used was beryllium fluoride in 20% aqueous solution. The detergents used were a standard enzyme detergent, as marketed, and the same detergent with enzyme omitted.*

Its main ingredients are anionic surfactants, sodium tripolyphosphate, sodium perborate, sodium silicate and sodium sulphate; minor ingredients (less than 1%) include perfume, colourings, optical brightener and enzyme. The enzyme is an alkaline protease, a subtilisin of the Carlsberg type, the enzyme ingredient containing 5–10% of the pure protease.

EXPERIMENT I

The method was based on that reported by Polak, Barnes & Turk (1968). The sensitizing application was made by painting 50 μl of the BeF_2 solution with or without detergent to the skin of the ear; the painting continued until all the solution was used up. This was repeated on three successive days.

The challenging dose of 1% BeF_2 solution was applied to the closely clipped skin of the flank 12–14 days after the last sensitizing application, and the response was observed for 2 days. These were the concentrations used by Polak *et al.* (1968) and the method of scoring was the same, i.e. 0 = no reaction, 1+ = red reaction but not raised, 2+ = red raised reaction.

Results

Group 1. 20% $BeF_2 \pm 1\%$ detergent (D) or 1% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	($n = 18$)
BeF_2	3	3	0	3/6
BeF_2 + ED	0	1	5	6/6
BeF_2 + D	0	0	6	6/6

Group 2. 20% $BeF_2 \pm 0{\cdot}5\%$ detergent (D) or 0·5% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	($n = 21$)
BeF_2	4	3	0	3/7
BeF_2 + ED	3	4	0	4/7
BeF_2 + D	1	6	0	6/7

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* Supplied by Proctor and Gamble Ltd, by courtesy of Mr L.T.Bowerman.

Conclusion

The presence of enzyme was not shown to increase the chance of sensitization.

EXPERIMENT 2

Pretreatment consisted of painting the ear with distilled water or with detergent solution for a given period, followed immediately by application of the sensitizer (20% BeF_2 without detergent) as in experiment 1. The procedure was repeated on three successive days and the challenge made 12–14 days later as in experiment 1.

Results

Group 3. 30 min pretreatment, 0·5% detergent (D) or 0·5% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	(*n* = 6)
Water	2	0	0	0/2
Water+ED	2	0	0	0/2
Water+D	1	1	0	1/2

Group 4. 30 min pretreatment, 1% detergent (D) or 1% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	(*n* = 12)
Water	2	2	0	2/4
Water+ED	3	1	0	1/4
Water+D	3	1	0	1/4

Group 5. 30 min pretreatment, 2% detergent (D) or 2% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	(*n* = 6)
Water	2	0	0	0/2
Water+ED	0	2	0	2/2
Water+D	1	1	0	1/2

Group 6. 15 min pretreatment, 1% detergent (D) or 1% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	(*n* = 24)
Water	2	6	0	6/8
Water+ED	3	5	0	5/8
Water+D	5	3	0	3/8

Group 7. 10 min pretreatment, 0·5% detergent (D) or 0·5% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	($n = 6$)
Water	0	2	0	2/2
Water + ED	1	1	0	1/2
Water + D	0	2	0	2/2

Group 8. 5 min pretreatment, 0·5% detergent (D) or 0·5% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	($n = 6$)
Water	0	2	0	2/2
Water + ED	0	2	0	2/2
Water + D	0	2	0	2/2

Group 9. 5 min pretreatment, 0·25% detergent (D) or 0·25% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	($n = 6$)
Water	1	1	0	1/2
Water + ED	1	1	0	1/2
Water + D	1	1	0	1/2

Group 10. 1 min pretreatment, 0·25% detergent (D) or 0·25% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	($n = 6$)
Water	1	1	0	1/2
Water + ED	0	2	0	2/2
Water + D	0	2	0	2/2

Group 11. 1 min pretreatment, 0·125% detergent (D) or 0.125% enzyme detergent (ED).

	Reaction			Proportion sensitized
	0	1	2	($n = 6$)
Water	1	1	0	1/2
Water + ED	1	1	0	1/2
Water + D	0	2	0	2/2

In groups 5, 6 and 7 a considerable primary irritant effect was observed in all cases, usually on the second or third day of pretreatment. The reaction was less after 15 min and shorter pretreatment periods, and appeared to be related to the duration of pretreatment rather than to the presence or absence of detergent or enzyme. Reactions were of the same degree when distilled water was used for pretreatment. Pretreatment with detergent solutions alone, without subsequent application of BeF_2, did not produce a primary irritant reaction.

Conclusion

Pretreatment considerably enhanced the development of primary irritant reactions; these were related to the duration of pretreatment and not to the presence of detergent or of enzyme. The presence of detergent or of enzyme detergent in the pretreatment solution did not promote sensitization.

EXPERIMENT 3

Animals were used from groups 1 and 2 or freshly sensitized by the same method. They were then patch-tested on three sites simultaneously, on the closely shaved skin of the flank using 1% BeF_2 solution alone or with the addition of 1% detergent (D) or of 1% enzyme detergent (ED).

Results

Group 12.

	Reaction			Proportion sensitized
	0	1	2	($n = 15$)
BeF_2	1	4	0	4/5
BeF_2+ED	2	2	1	3/5
BeF_2+D	3	2	0	2/5

From Group 1.

	Reaction			Proportion sensitized
	0	1	2	($n = 54$)
BeF_2	4	10	4	14/18
BeF_2+ED	6	10	2	12/18
BeF_2+D	5	8	5	13/18

From Group 2.

	Reaction			Proportion sensitized
	0	1	2	($n = 45$)
BeF_2	5	10	0	10/15
BeF_2+ED	1	11	3	14/15
BeF_2+D	1	11	3	14/15

Conclusion

The incorporation of enzyme detergent or of detergent alone in the test solution does not promote positive patch-test reactions in animals previously subjected to a sensitizing prodecure.

Numbers in the various groups are in some cases small, but throughout these experiments the effect of detergent in promoting positive results emerges from time to time. There has, however, been no combination of sensitizer and detergent in which the presence of enzyme appears to exert any such effect.

We have, therefore, been unable to find, by this mechanism, any support for a suggestion that the incorporation of this enzyme in a detergent leads to an increased risk of skin sensitization or irritation.

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Keratinization in dispersed cell cultures of adult guinea-pig ear skin

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SUMMARY

A study of the behaviour of dispersed cell cultures of adult guinea-pig keratinocytes has been made using time-lapse cinephotomicrography and electron microscopy. Attention has been directed mainly to the formation of aggregates and the ultrastructure of the cells involved. At the periphery of the clumps and at the edges of spaces within the clumps the cells were organized in a layered system and fully keratinized cells occurred only within a three dimensional system. Some partial keratinization and some autolysis was also noted.

Cruickshank, Cooper & Hooper (1960) described a method for studying cells from the adult epidermis in dispersed cell culture. This was based essentially upon the use of a special chamber suitable for long term culture and compatible with good phase contrast cinephotomicrography. The method with various minor modifications has since been used with success to study the behaviour of keratinocytes and their interactions with melanocytes (Cruickshank & Harcourt, 1964; Cohen & Szabo, 1968; Klaus & Snell, 1967; Fritsch & Diem, 1972).

This communication reports the results of further studies on the behaviour of keratinocytes using time-lapse cinephotomicrography and electron microscopy.

MATERIALS AND METHODS

Only minor modifications of the original method have been made. Instead of perspex or glass chambers, similar disposable chambers made of tissue culture quality plastic were used (Sterilin Ltd). The medium was 30% serum (usually guinea-pig or calf) and 70% of Eagle's MEM containing 1% L-glutamine (200 mmol) and penicillin and streptomycin each 100 iu/ml. It was buffered with Hepes (Sigma London Chemical Co.) as described by Fisk & Pathak (1969).

The guinea-pig ear slices were trypsinized in 0·25% trypsin containing amphotericin B for 20 min, and after separation the epidermis was replaced in trypsin for a further 10 min with occasional brisk agitation. The pieces of horny layer were removed by forceps and the cell suspension centrifuged at 600 *g* for 3 min and then resuspended in culture medium at a density of about 10^3 cells/mm^3.

On the third day of culture, medium and non-adherent cells were removed. To this medium 25% fresh medium was added and the whole filtered through a millipore filter (0·45 μm) and replaced in the vessel. Subsequent medium changes were made twice-weekly. Cells growing in Cruickshank chambers were filmed by time-lapse cinephotomicrography.

For electronmicroscopy the cells were grown in Falcon flasks (Becton Dickinson & Co. Ltd), fixed and dehydrated *in situ* according to the method of Constable (1972). Suitable clumps of cells were selected by phase contrast microscopy and their position marked on the outside of the flask with a felt tipped pen. After dehydration the top of the flask was completely removed with a hot scalpel and the cells were impregnated with three changes of Epon. The final change of Epon was decanted leaving a very thin layer which was then partially polymerized. The closed ends of 5 × 50 mm nylon centrifuge tubes were cut off and the resulting open tubes were placed over the marked areas and the flask with tubes was replaced in the incubator for a further 24 h. The empty tubes were filled with Epon which was subsequently polymerized. The flask and Epon were chilled with solid carbon dioxide and the columns of Epon were snapped off from the plastic flask. The clumps of cells could be seen and the surface of the column trimmed to provide a block face from which ultrathin sections were taken. Sections were collected on uncoated copper grids, stained with saturated methanolic uranyl acetate and lead citrate (Reynolds, 1963) and were viewed in a Siemens Elmiskop 1A.

For ultrastructural examination several complete clumps were sectioned right through and sections examined from every successive level, commencing with those on the substrate.

RESULTS

As seen by phase contrast and cinephotomicrography immediately after inoculation, the cells are rounded and discrete. They are adherent to the substrate within a few hours but by 24 h they tend to form aggregates of from two to twelve cells (Fig. 1a, b). These adhere to the substrate by cells on the periphery which show ruffling movements. The clumps are moved by these peripheral cells and at times appear to move positively towards each other. They may remain united to form larger and multiple clumps or may move apart again. Aggregates visible to the naked eye clearly in three dimensions may form from the second or third day onward. During this period the behaviour pattern of cells in the same culture varies. Non-aggregated cells may divide to initiate monolayer colonies; cells may crawl out from the clumps and behave similarly or clumps may remain as such for several days showing, as judged by phase contrast, little activity apart from ruffling of the peripheral membranes. Apart from the initial aggregates which keratinize, those cells which have formed monolayers also tend to aggregate into clumps, sometimes in the middle of the colony (Fig. 1c) but also at the periphery, in which situation the aggregate has the form of a straight edge.

As seen by electronmicroscopy, cells nearest the substrate were healthy and were similar to those in monolayers previously described by Constable (1972). Within the clumps, where the cells were arranged in three dimensions, several novel features were found. At the edges, where the clump bordered on the culture medium, the appearance was reminiscent of cells grown in organ culture (Fig. 2a). The surface cells were elongated and flattened and connected to each other by small desmosomes and villous processes. In places where a cell process seemed to form a leading edge, the cytoplasm contained many pinocytotic vesicles, microfilaments and microtubules, and the plasma membrane was ill-defined (Fig. 2b).

Within the clumps there were spaces and bordering on to these the cells were again organized in layers of flattened cells. The spaces were apparently filled with material which was precipitated during processing and into this material projected many small microvilli (Fig. 3a, b, c). Those cells nearest the spaces were normal and healthy and sometimes contained keratohyalin granules in addition to

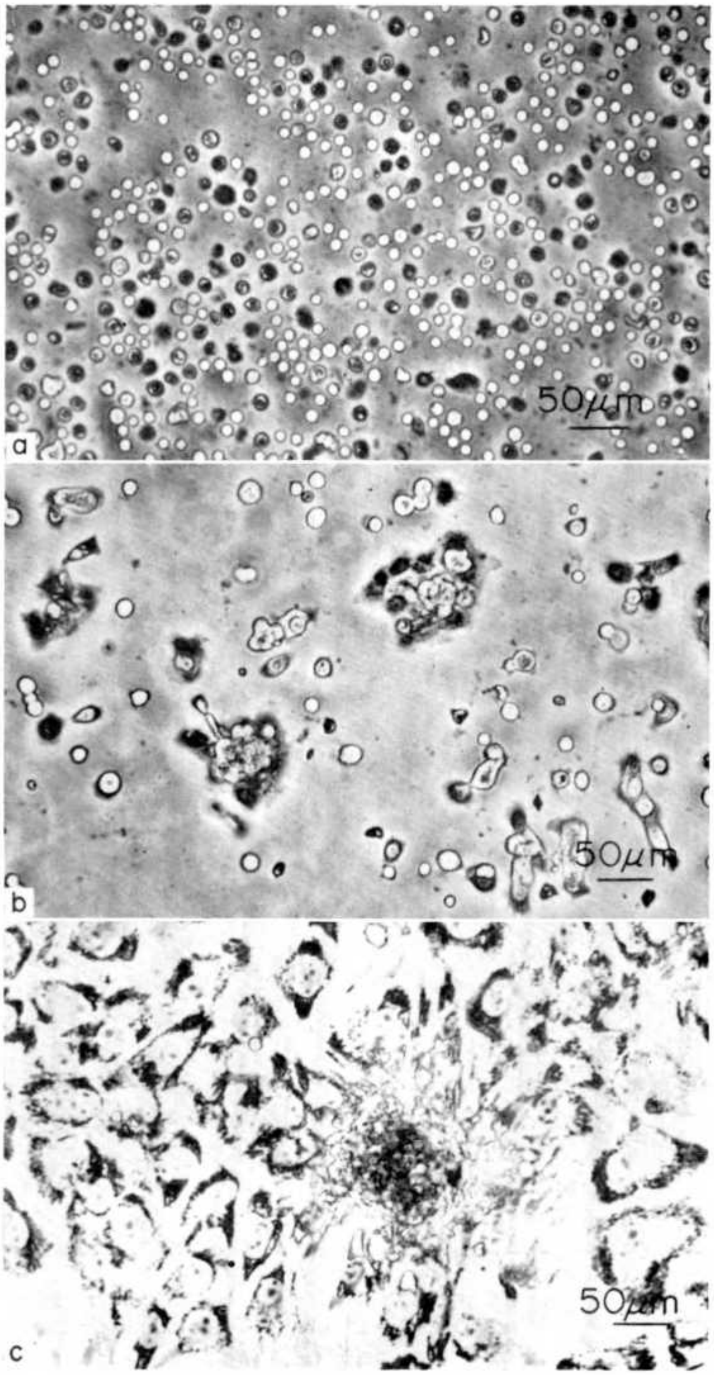

FIGURE 1. (a) Trypsinized cells 4 h after inoculation (× 160). (b) Early clump formation 12 h after inoculation (× 160). (c) Clump formation from monolayer colony—9 days (× 160).

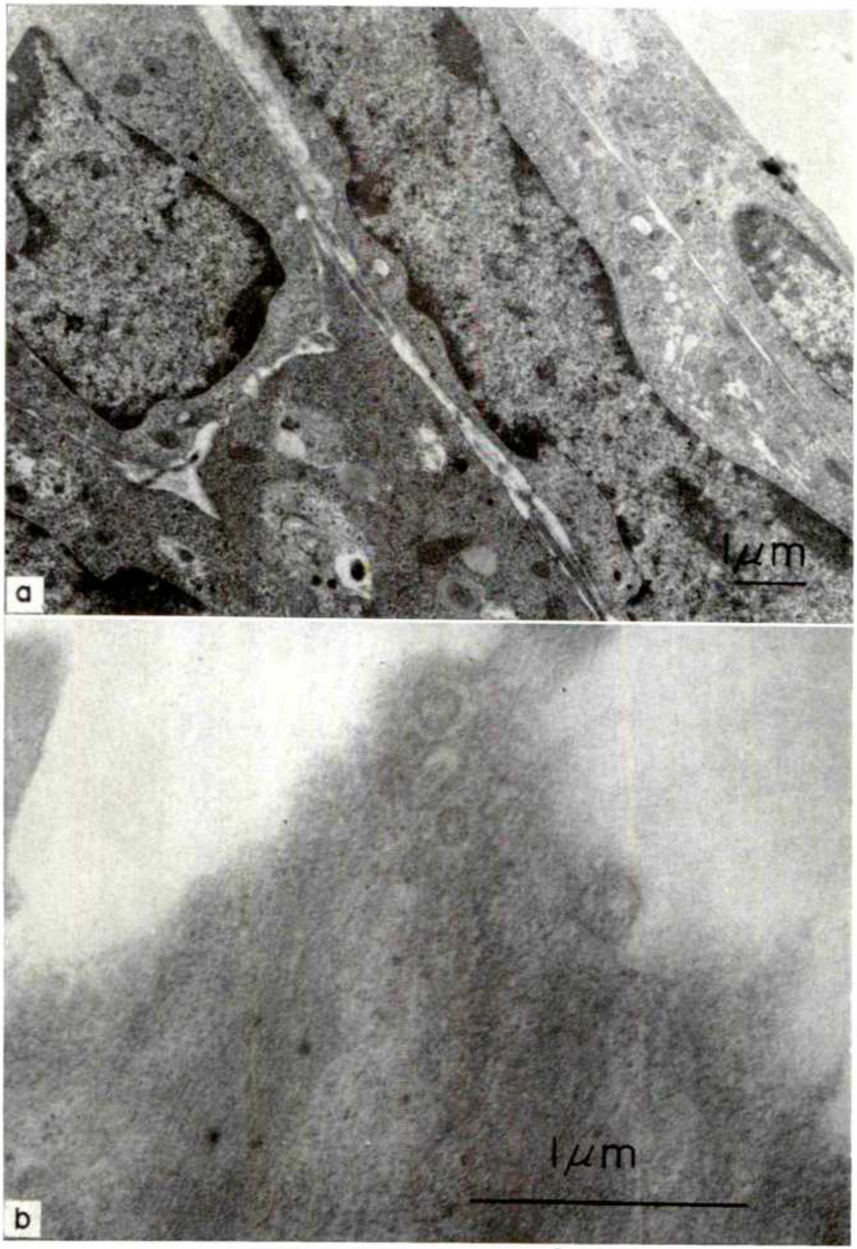

FIGURE 2. (a) Outer edge of a clump of cells showing flattened cells at the periphery (× 8700). (b) Leading edge of a cell showing pinocytotic vesicles, microfilaments and microtubules (× 36,400).

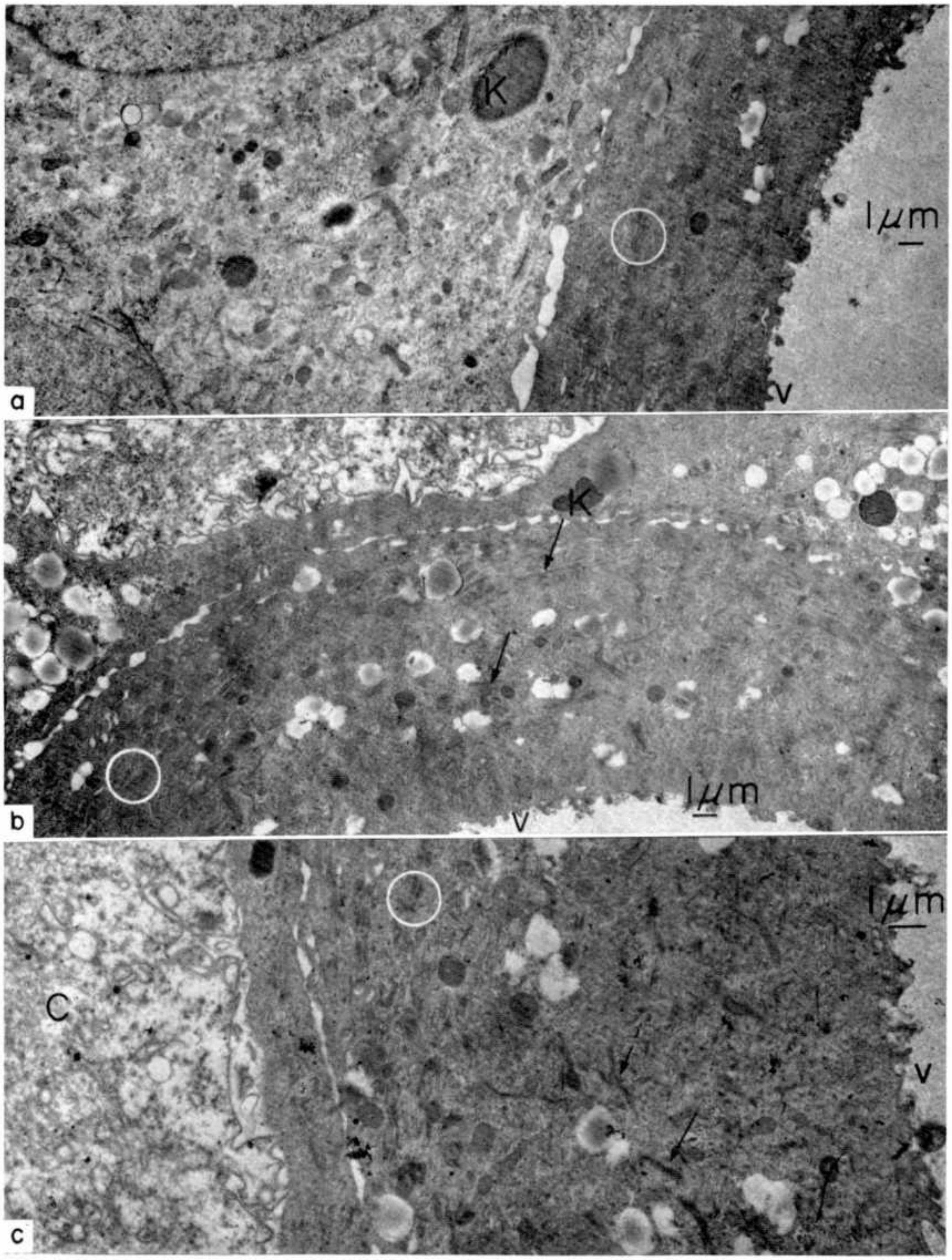

FIGURE 3. Cells arranged in layers around a hole within the clump. Microvilli (v), keratohyalin (k), desmosomes (○), tonofilaments (arrow), keratinizing cell (C). (a) (× 4300); (b) (× 4400); (c) (× 5800).

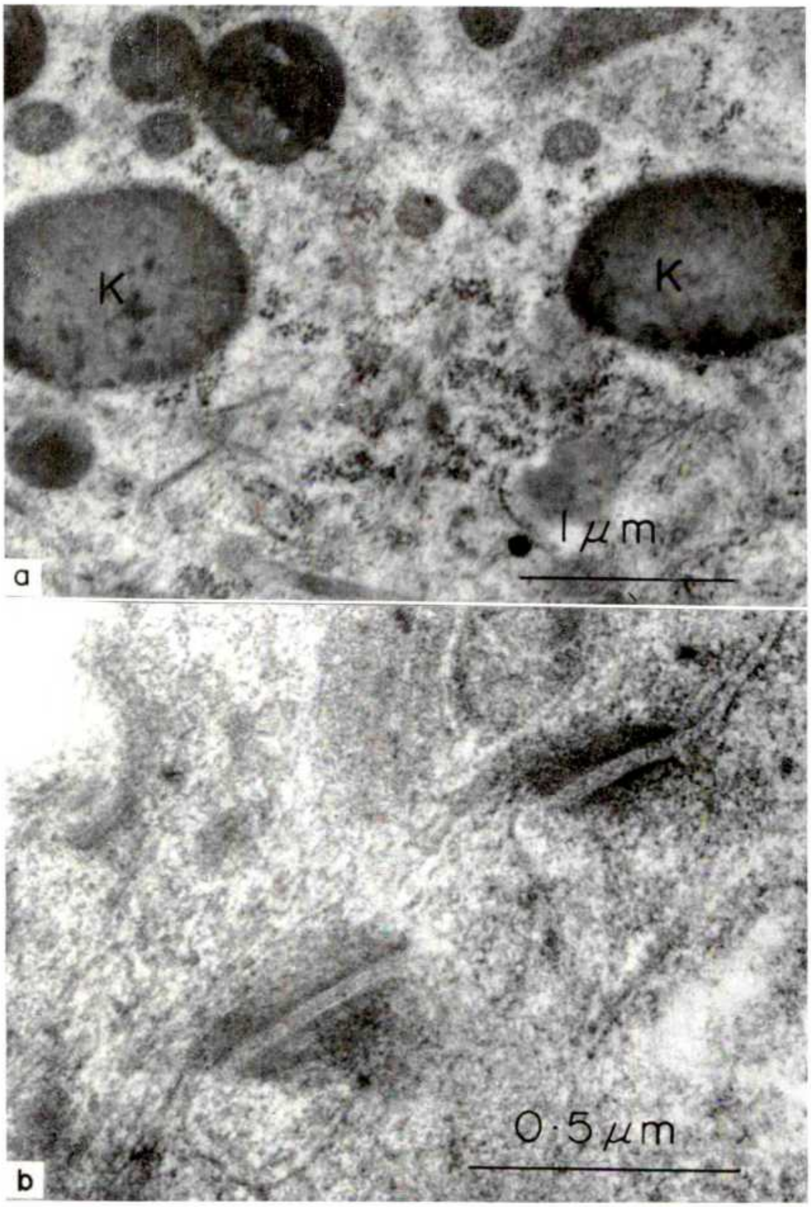

FIGURE 4. (a) Keratohyalin granules (K) in a flattened cell (× 28,600). (b) Desmosomes between layered cells (× 69,000).

tonofilaments (Figs. 3a, b, and 4a). Cells in these areas possessed many desmosomes (Figs. 3 and 4b). At a variable distance from this internal edge many of the cells developed thickened membranes, lost intracellular organelles and acquired a fibrous appearance suggesting that they had undergone at least partial keratinization (Fig. 3a, c).

Some layered cells showed a progression from healthy, through a finely fibrous stage with thickened

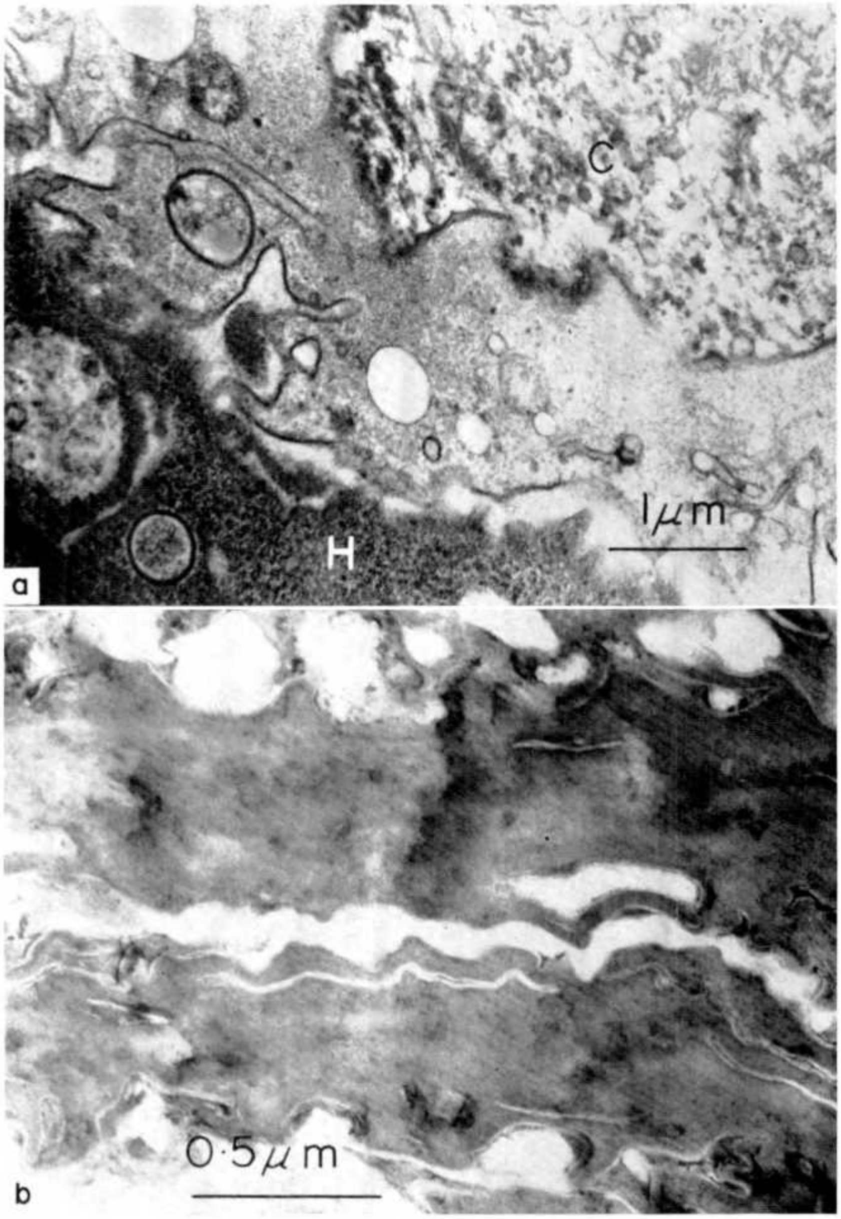

FIGURE 5. (a) Layered cells showing a progression from healthy cell (H) to fibrous partly keratinized cell (C) (× 16,800). (b) Fully keratinized cells in layers (× 50,800).

plasma membrane, to the type of 'open' fibrous cell already described (Fig. 5a). In other areas, fully formed interlocking squames were seen (Fig. 5b).

The cells between these organized areas were much more rounded and obviously randomly orientated in all three dimensions (Fig. 6a, b). There was great variability amongst the cells. Some were relatively normal with indented nuclei, elongated mitochondria and polyribosomes, which are all

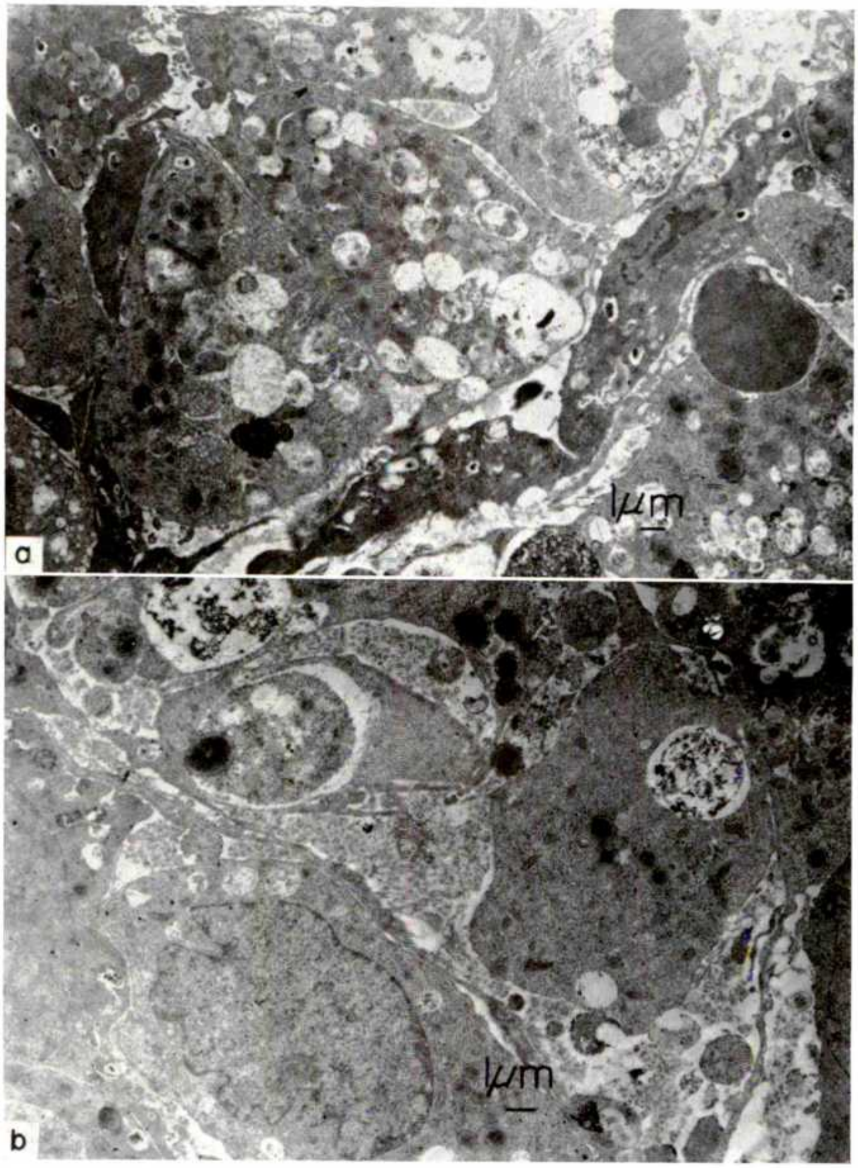

FIGURE 6. (a) Randomly orientated cells of varying electron density. Lysosomes are prominent (× 3000). (b) Randomly orientated cells. Relatively healthy cells are interspersed with heavily vacuolated cells and cell debris (× 3000).

characteristic of cells in culture (Fig. 6b). Others were more electron dense than normal but appeared otherwise fairly healthy (Fig. 6a). Numerous lysosomes were prominent in all the cells in these areas and debris between cells indicates that some cells had died and undergone autolysis (Fig. 6a, b).

DISCUSSION

Our previous descriptions of epidermal cells in dispersed cell cultures have concentrated on those cells which form monolayers in culture and are thus arranged essentially in two dimensions (Cruickshank *et al.*, 1960; Constable, 1972). In the original observations the significance of the clumping of cell suspensions was not appreciated and by changing the medium at 24 h many clumps which had not become firmly attached to the substrate were removed. These were considered to be dead cells which might mitigate against the survival of the cells spreading on the substrate. This study has shown, however, that the clumping of cells is not followed by their death, but results in a degree of organization far more complex than is seen in the monolayer areas of the cultures. Those cells in the clumps which were still healthy 9 days after setting up the culture possessed many of the features of cells in monolayer culture, as described by Constable (1972), having long mitochondria, many polyribosomes, fat droplets, indented nuclei, etc. The numbers of tonofilaments and desmosomes in a cell appeared to depend on its position within the clump. Those cells which bordered, or were near a space, had many tonofilaments and desmosomes, whilst those further away had very few. The level of organization seen amongst cells which abutted on to a space was quite impressive whether the space was at the edge of a clump or was a hole within a clump. In both these situations the cells formed layers of flattened cells which were very reminiscent of cells undergoing epiboly in organ culure. This kind of arrangement is probably imposed to a certain extent by the rounded nature of the clump and the tensions produced by the physical connections, lateral and vertical, between neighbouring cells. Those cells which were in a layered part of the clump were apparently able to keratinize much more readily than the others. It was in layered cells that keratohyalin was seen, and those cells which had an open fibrous appearance, suggestive of partial keratinization, were usually situated very close to the layered cells. The fully keratinized squames were also layered and looked identical to keratin seen *in vivo*. The variable appearance of cells which were undergoing keratinization is not surprising in an *in vitro* situation where the cells are growing immersed in an artificial fluid medium. No keratinosomes were seen in either keratinized cells or in those containing keratohyalin, although it cannot be concluded from this that they were absent throughout the clumps. It does indicate, however, that they need not be present in significant numbers for keratinization to take place.

Those cells which were not adjacent to an edge showed a conspicuous absence of spatial or behavioural organization, and only occasionally was a possibly keratinized cell seen within this jumbled mass. Many of the cells contained numerous lysosomes which suggests that autolysis was taking place, as does the debris present between the cells. This apparent breakdown of the cells may be related both to the distance from a source of nutrients and to the evident lack of organization between the cells. It thus appears that the cells need considerable spatial organization in order to attain their keratinizing potential and without this organization ultimately autolyse and die.

These observations are consistent with the findings of other workers who have induced three-dimensional growth in epidermal cells under other conditions. Karasek & Charlton (1971), for example, grew epithelial cells on a collagen gel where they did not initially clump but formed a monolayer. Having done this they grew into the gel and on top of each other, finally forming a keratinizing epithelium. More recently, Bauer & De Grood (1973) have induced aggregation by adding nucleoprotein to cell suspensions, and after subculturing under organ culture conditions a keratinizing epithelium was formed.

The possible role of the Langerhans' cell in the organization of the keratinocytes is not clear. There is some suggestion from current histochemical studies that it may be involved in the formation of clumps, but we have not so far found any in those clumps examined in the electron microscope and this question must await further investigation.

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The activity of various topical griseofulvin preparations and the appearance of oral griseofulvin in the stratum corneum

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SUMMARY

Stratum corneum may be stripped onto a tape, sterilized and then inoculated with *Trichophyton mentagrophytes* spores. Under controlled conditions the spores germinate *in vitro* in the stratum corneum. This method has been quantified and adapted to the investigation of topical antifungal agents. Various formulations of topical griseofulvin were tested for activity and duration of effect with this method. The antifungal effect of 1% griseofulvin in a cream base was weak and of short duration. 1% griseofulvin in dimethylacetamide (DMAC) was totally effective for 24 h and could be detected 96 h after one application. Griseofulvin in DMAC was also totally effective, as a prophylactic agent, against experimental human *T. mentagrophytes* infections for up to 72 h after one application.

Griseofulvin in an oral dose of 1 g daily was detected in all three layers of the stratum corneum within 8 h of administration.

Two techniques have been utilized in the study of topical and systemic griseofulvin. The first is the use of standard reproducible experimental *Trichophyton mentagrophytes* infections in humans (Knight, 1972a) and the other is an *in vitro* system for the culture of dermatophytes on human stratum corneum (Knight, 1972b).

MATERIALS AND METHODS

Griseofulvin powder was used to prepare the following topical preparations: 1% griseofulvin in dimethylacetamide (DMAC), 1% griseofulvin in Aquacare cream, 0·1% griseofulvin in DMAC and 0·1% griseofulvin in ethyl alcohol. Microcrystalline griseofulvin 0·5 g tablets were used for oral administration.

In vitro *assay for antifungal compounds in stratum corneum*

This method has already been described in detail (Knight, 1973). Stratum corneum is stripped off onto a piece of translucent adhesive tape by repeated application directly to the skin. It is then placed, stratum corneum surface uppermost, onto a microscope slide and fixed at each end by small pieces of the adhesive tape. The slide is placed on a bent glass rod in a petri dish and sterilized in ethylene oxide gas. Later, water is run into the bottom of the dish and a spore suspension of *T. mentagrophytes* is applied to the centre of the tape and spread. The cover is replaced and the culture is incubated at 30°C for 7 days. Microscopic observations show a mass of hyphal filaments on the stratum corneum after incubation. If the stratum corneum contains an anti-fungal compound then the degree of growth is reduced and in the presence of larger concentrations only short ballooned germinal tubes are produced. Based on these observations a system of grading for the tape cultures was devised. Grade 0 was the production of short ballooned germinal tubes only. Grade 1 was the presence of hyphal filaments which were short; only crossing from one to two scales of stratum corneum. Grade 2 was characterized by the area of inoculation being covered by long straight filaments; this was typical of normal stratum corneum. Grade 3 had in addition a few true thalli.

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Volunteers used an inert soap for 2 weeks prior to and during all investigations. One anti-fungal compound only was used at a time. Separate areas 9 cm × 5 cm were marked out on the upper arms. Stratum corneum strippings were taken from the first area prior to application of the drug. To the other areas the anti-fungal compound was applied and left for 15 min. The areas of application were then washed with a standard procedure and dried. Strippings were taken from one area at each specified time interval. After sterilization, inoculation with spores, and incubation, the tape cultures were graded in a double-blind fashion. Four subjects were utilized for each compound and four strippings were taken from each site.

There were two series of experiments performed for the detection of griseofulvin in the stratum corneum after oral administration. During the first experiment, eleven human volunteers took 0·5 g of griseofulvin each morning, following a light breakfast, for 36 days. One stratum corneum stripping was taken daily for 5 days and at intervals thereafter. The second experiment was performed in order to obtain more detailed information by more frequent sampling. Strippings were taken from the top, middle and lower layers of stratum corneum. The two lower layers were reached by repeated stripping with Scotch tape. Griseofulvin tablets 1·0 g daily were taken by each subject starting at zero time, immediately after the control stripping samples. The last dose was taken after 72 h. Three strippings were taken from each subject at zero time, 8 h, 24 h and at intervals thereafter for 8 days. Six subjects took part in the investigation.

Experimental human T. mentagrophytes *infections*

Experimental infections were produced by the application of 10,000 spores to the upper arms of eighteen volunteers and occluding for 72 h. Four inoculations were made on each arm. One arm acted as the control and the other had four areas marked out to which 1% griseofulvin in DMAC was applied and left for 15 min. The areas were washed in a standard fashion and inoculations were made to both arms after 24, 48, 72 or 96 h of normal activity. The sites were examined 72 h after removal of the dressings. A positive infection was one which was either culturally or microscopically positive.

RESULTS

The results of the topical griseofulvin *in vitro* experiments are summarized in Table 1. The numbers indicating the degree of growth are the sums of the gradings from sixteen tape strippings, four from each of four subjects. Most pre-treatment scores were two for each tape stripping. 1% griseofulvin in DMAC was the most potent and long lasting preparation. There was almost complete inhibition of fungus growth for up to 96 h after one application. 1% griseofulvin cream was much less active and was not detectable after 24 h. There was a statistical difference between the 0·1% formulations of

TABLE 1. A comparison of the effectiveness of various formulations of topical griseofulvin as shown by inhibition of *T. mentagrophytes* spore growth on stratum corneum *in vitro*

Time (h)	Degree of growth			
	1% Griseofulvin in DMAC	1% Griseofulvin cream	0·1% Griseofulvin in DMAC	0·1% Griseofulvin in alcohol
Pretreatment	34	32	32	35
0 h	0	5	5	22
Probability	$P<0{\cdot}01$	$P<0{\cdot}01$	$P<0{\cdot}01$	NS
8 h	1	10	13	27
Probability	$P<0{\cdot}01$	$P<0{\cdot}01$	$P<0{\cdot}05$	NS
24 h	3	23	19	33
Probability	$P<0{\cdot}01$	NS	NS	NS
48 h	4	32	24	35
Probability	$P<0{\cdot}01$	NS	NS	NS
72 h	13	33	26	37
Probability	$P<0{\cdot}05$	NS	NS	NS
96 h	14			
Probability	$P<0{\cdot}05$			
120 h	21			
Probability	NS			

griseofulvin in DMAC and in ethyl alcohol up to 24 h ($P<0{\cdot}05$). DMAC, Aquacare cream and ethyl alcohol had no anti-fungal effect, when used alone, with this method.

Only 1% griseofulvin in DMAC was tested for a prophylactic effect against human experimental *T. mentagrophytes* infections (Table 2). One short exposure was found to be completely effective for 72 h.

Two series of experiments were conducted for the detection of griseofulvin in stratum corneum after oral administration. In Fig. 1 each point represents the sum of the degree of growth obtained by grading eleven tape strippings (one from each of eleven volunteers). The degree of growth dropped by

TABLE 2. Results of human experimental *T. mentagrophytes* infections after prophylactic use of 1% griseofulvin in DMAC

Time (h)	No. of positive infections	
	Normal sites	Griseofulvin pretreated
24	11/12	0/12
48	11/12	0/12
72	24/24	0/24
96	24/24	5/24

At all times there was a statistical difference ($P<0{\cdot}01$). Probability assessed by Student's '*t*' test.

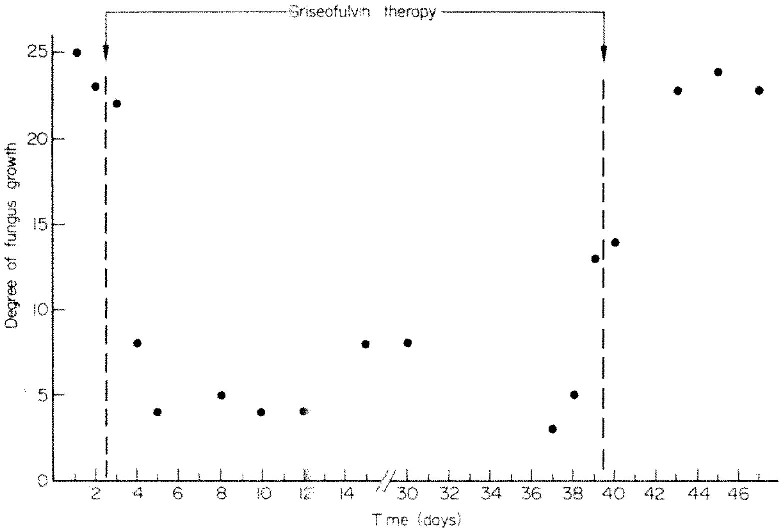

FIGURE 1. Tape strippings from eleven patients started on griseofulvin 0·5 g/day.

50% within 48 h of oral administration of the drug. It was maintained at this level until the first sample after stopping the drug, 4 days later. The results of the second experiment are shown in Fig. 2. This shows the total scores of the grading from three tape strippings from each of six volunteers at specific time intervals. The maximum score at each point is thus 54. There was a decrease in the degree of

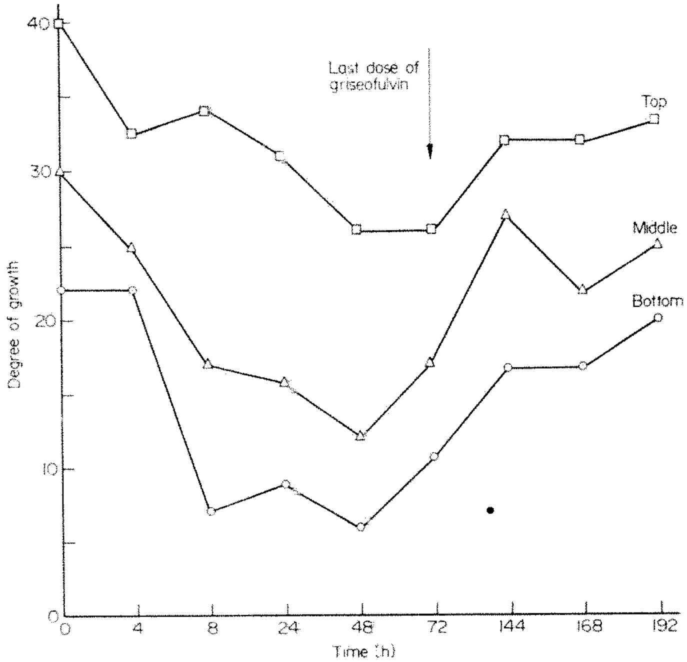

FIGURE 2. Stratum corneum strippings from six patients starting on griseofulvin tablets 1·0 g daily.

growth in the upper and middle layers as early as 4 h and in all three layers at 8 h after oral griseofulvin. The lowest point was reached at 48 h. There was an increase in the degree of growth towards normal after stopping the drug. An analysis of variance (*F*-test) was performed on this data. Scores were significantly different at different times ($P<0{\cdot}01$). Results for the three layers were not significantly different ($P<0{\cdot}25$). The *t*-test was also used to determine whether the means were significantly different from zero. By 8 h there was a significant degree of inhibition of growth in the top layer ($P<0{\cdot}05$), in the middle layer ($P<0{\cdot}02$), and in the bottom layer ($P<0{\cdot}01$). By 48 h the degree of significance for all layers was $P<0{\cdot}01$. Four days after stopping the drug its effect had gone from the middle and lower layers but was still present in the upper layer ($P<0{\cdot}05$).

An attempt was made to determine the approximate concentration of griseofulvin in the stratum corneum after oral therapy, by comparison with stratum corneum tape strippings dipped in various concentrations of griseofulvin in 70% ethyl alcohol. There was approximately 50% inhibition of growth after dipping in 10 μg/ml of griseofulvin, this being similar to that found after oral therapy.

DISCUSSION

Numerous investigators have used topical griseofulvin in clinical trials and noted improvement in treated lesions. Martin (1959) used milled griseofulvin tablets to make up a 0·1% solution in arachis oil. He reported some suppression of treated lesions when compared with control subjects. Goldman, Preston & Schwarz (1959) used 0·5% and 1% lotions in a monoglyceride vehicle. They noted improvement with therapy but a high relapse rate following cessation of the drug. Griseofulvin in an ointment base with salicylic acid has been shown to be more effective. Höfer, Hönemann & Koch (1968) used 5% griseofulvin with 3% salicylic acid in a cream base to treat sixty patients with dermatomycoses. Within 21 days, 83% of lesions were clinically and mycologically clear. Others have reported favourable results after therapy with griseofulvin in a cream base with salicylic acid (Wozniak, Friedrich & Janik, 1970; Arievich & Ziserman, 1972). Levine, Cobb & Friedman (1971) published some pertinent data on the penetration of griseofulvin in dimethylsulphoxide (DMSO) into guinea-pig skin. After washing the skin thoroughly they were able to detect inhibitory concentrations of griseofulvin, in subkeratinous layers of guinea-pig skin, after topical application of 25% griseofulvin in DMSO. They were able to detect 1–4 μg of griseofulvin per gram of tissue. Also the same topical preparation was used to treat *Microsporum canis* infections in eleven cats. With one daily application for 10 days all cleared. The disease in thirty untreated cats was unchanged after 10 weeks. DMSO alone failed to improve the lesions. A suspension of griseofulvin in saline (250 mg/ml) was used topically on infected cats with no improvement. Griseofulvin is relatively insoluble in water but readily soluble in DMSO and DMAC. The latter solvents generally enhance penetration (Stoughton & Fritsch, 1964; Munro & Stoughton, 1965) and have been shown to specifically increase penetration of griseofulvin. This point is well illustrated by comparing the results of the *in vitro* investigations using 1% griseofulvin in DMAC and 1% griseofulvin in Aquacare cream. At zero time, after washing the area, there is complete inhibition of growth by griseofulvin in DMAC, and it is still detectable after 5 days of normal activity. However, in the cream base, 1% griseofulvin does not give complete inhibition even at zero time and has no significant effect after 24 h. It seems that the cream does not allow penetration of the griseofulvin into the stratum corneum and is readily washed off the surface of the skin.

The method used for the detection of griseofulvin in the stratum corneum in this investigation depends upon the effect of griseofulvin on the growth of *T. mentagrophytes* spores. Brian, Curtis & Hemming (1946) were the first to observe abnormalities produced by the antibiotic in the germ tubes of *Botrytis allii*. Roth, Sallman & Blank (1959) investigated the concentrations necessary to produce these abnormalities and in the following year Roth & Blank (1960) used a bio-assay system, based on these observations, to detect griseofulvin in human stratum corneum after oral administration. The stratum corneum was stripped to the required depth and macroaleuriospores of *M. gypseum* were applied. Agar was run into a ring over the spores and kept in position for 14–16 h. The microculture was removed, stained with lactophenol cotton blue dye and the germ tubes examined. The assay thus depended upon the ability of the griseofulvin to diffuse into the agar. They found evidence of griseofulvin in the base of the stratum corneum within 72 h after ingestion. However, it took 7–56 days for it to be detected in the mid stratum corneum and it was never detected on the surface. The conclusion was that griseofulvin was incorporated into the stratum corneum by a metabolic process.

Recently, Epstein, Shah & Riegelman (1972) used a gas–liquid chromatographic procedure and electron capture detector to assay griseofulvin in stratum corneum. Stratum corneum was curetted off from three distinct levels, and griseofulvin estimations were made on specimens weighing 1–3 mg. Griseofulvin was detected in palmar skin within 8 h at all three levels. Concentrations in the stratum corneum were higher per gram of tissue than in plasma, thus the griseofulvin could not be simply diffusing into the stratum corneum from the plasma. There was also more griseofulvin detected in the superficial stratum corneum than in the deeper layers. This was explained by the suggestion that griseofulvin was dissolved in the sweat and deposited in the horny layer during evaporation of the water.

CONCLUSIONS

Evidence has been presented to show that griseofulvin is a very potent topical anti-fungal compound in DMAC. Its potency is less in other vehicles. In view of this it may be necessary to review the current attitude to topical griseofulvin therapy.

Griseofulvin detection in the stratum corneum within 24 h of oral administration is evidence against the theory of a metabolic incorporation.

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The investigation of superficial fungal infections by skin surface biopsy

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SUMMARY

The skin surface biopsy technique using a cyanoacrylate contact cement was employed for the diagnosis and study of superficial mycoses. The biopsy specimens were used for quick direct examination after treatment with potassium hydroxide and blue-black ink, direct examination after staining with the periodic-acid Schiff method, fungal culture and scanning electron microscopy.

Skin surface biopsies were taken from 124 patients for one or more of these purposes. Direct examination and culture of the specimens proved simple, and reliable for diagnostic use in clinical practice. In addition, the morphology of the infective agent could easily be studied in detail in its *in vivo* arrangement in the intact sheets of horny layer, either in stained preparations or under the scanning electron microscope.

The causal agent of a superficial mycosis is generally identified by direct examination and culture of skin scrapings; these are often difficult to collect and tend to be blown away by the slightest breath of air. The small keratin fragments show little of the growth patterns of superficial fungi, which proliferate largely in a horizontal plane. Other methods may be employed for more extensive study of intact sheets of the stratum corneum and include adhesive tape strippings (Keddie, Orr & Liebes, 1961; Keddie & Sakai, 1965), glass slides with a sticky coating (Goldschmidt & Kligman, 1967), and skin replication techniques (Sarkany, 1962; Sarkany & Caron, 1965; Bernstein & Jones, 1969).

A good method for the diagnosis of a superficial mycosis should combine ease of collection with simple processing for direct examination and culture. The technique of taking a skin surface biopsy (Keddie *et al.*, 1963; Marks & Dawber, 1971) suits these requirements and allows the ready removal of large areas of intact horn cells without much discomfort to the patient. The cells, being cemented firmly to the glass slide, are already mounted for examination and cannot be lost or deteriorate to any extent even if there is a delay in transit to the laboratory. These unfixed specimens are suitable for a variety of staining techniques (Marks, 1972), the periodic-acid Schiff (PAS) method having already been used to demonstrate fungal structures (Keddie *et al.*, 1963; Keddie & Shadomy, 1963; Marks & Dawber, 1972). More detailed studies have been made using the scanning electron microscope (Marks & Dawber, 1971, 1972; Dawber, Marks & Swift, 1972).

The skin surface biopsy method was adopted and studied here. The original intention was to assess its reliability, combined with the use of potassium hydroxide (KOH) and ink, as a quick diagnostic aid in the consulting room. In a number of cases, findings with this technique were checked by direct examination of a PAS-stained specimen in the laboratory. Later on, specimens were taken to see whether they were suitable for fungal culture, and where possible, four surface biopsies were collected from each patient so that scanning electron microscopy (SEM) could be done as well. Results in 124 cases have proved satisfactory and are described here.

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* Reprint requests to: 801, Lancet Hall, Jeppe Street, Johannesburg, South Africa.

METHODS

An active skin lesion was selected and cleaned with acetone. A few drops of alpha-cyanoacrylate contact cement* were placed on the area and a clean glass slide pressed firmly on top of them. The cement polymerizes rapidly in the presence of pressure and traces of moisture, thus the slide could be peeled off the skin after approximately 30 s, with several layers of cells and even hairs glued firmly to it. The configuration of the lesion was preserved without distortion on the continuous sheet of stratum corneum obtained. For obvious reasons the method was unsuitable for collection of specimens from interdigital spaces and with a few exceptions adhesion was inadequate to obtain strippings from palms and soles and greasy or macerated skin. Satisfactory biopsies could usually be taken from other parts of the limbs and from the trunk and face, and surprisingly good specimens of scalp hair were obtained from cases of tinea capitis.

Quick direct examination with KOH and ink

The slide was flooded with a mixture of two parts 20% KOH and one part blue–black ink† for 5 min. It was then firmly blotted with a folded tissue and examined microscopically under good light with the substage condensor diaphragm wide open. Bluer, heaped-up patches of cells were examined first, as these often indicated active parts of the lesion, and hyphae could be found more quickly.

PAS stain

It became routine first to parcel the biopsy slide with fine sewing cotton because the skin often became detached from the glass during processing. The specimen was fixed in absolute alcohol for 1 min, flooded with 1% periodic acid for 5 min followed by Schiff's reagent for 20 min, and finally counterstained with light green for 15 s. After washing with distilled water, dehydration with increasing concentrations of alcohol and clearing with xylene, it was mounted in Depex‡ and a coverslip applied.

Fungal culture

The slide was laid face down on Actidione agar. This medium, consisting of Sabouraud's dextrose agar, cyclohexamide and chloramphenicol, was chosen to try to minimize the growth of aerial contaminants. After incubation at 25°C for 2 or 3 days, the slide with its attached specimen was moved with sterile forceps to a second Atidione agar plate. Both plates were incubated for up to 3 weeks and examined macro- and microscopically every few days for growth.

Scanning electron microscopy

An area of the surface biopsy which appeared to be actively diseased was selected and the slide was broken away to leave a specimen of about 1 cm diameter. This was cemented onto the aluminium scanner stub with colloidal graphite in alcohol* and coated with silver at 10^{-4} to 10^{-5} Torr at 20 kV in an Edward's Coating Unit with a special rotator-tilt fitting. The specimen was examined with a Cambridge Stereoscan S4 scanning electron microscope at 20 kV, and viewed at a suitable angle.

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* Permabond, Millner's Dental Suppliers, Cape Town.

† Quink, Parker.

‡ Depex mounting medium, George T.Gurr Ltd, U.K.

DISCUSSION

Quick direct examination with KOH and ink

The specimen was taken and processed early in the patient's examination so that it was ready towards the end of the consultation. The KOH allowed sufficient clearing of keratin in 5 min for fungal structures to become visible. Treated slides were found unsuitable for examination much later in the day as they deteriorated and became obscured by the formation of KOH crystals and by general darkening of the specimen. Early in the trial paired comparisons between skin surface biopsies and conventional skin scrapings showed the former method to be no less reliable than the latter.

TABLE 1. Direct examination with KOH and ink

Condition	No. of cases	Positive results
Tinea corporis	21	21
Tinea cruris	15	14
Tinea capitis	5	5
Tinea pedis	2	1
Pityriasis versicolor	54	52
Candidiasis	24	15
Erythrasma	2	0
Tinea nigra	1	1
Totals	124	109

Direct examinations with KOH and ink were done on surface biopsies from 124 patients (Table 1). Fungal structures were found in 109 of these cases. It can be seen that a high degree of accuracy was obtained in diagnosing cases of tinea corporis, cruris and capitis, and of pityriasis versicolor. The two negative results in pityriasis versicolor were in patients with pale and non-scaly lesions which were probably inactive at the time of examination. Candidiasis was much more difficult to diagnose; the pseudohyphae are thinner and harder to find than dermatophytic hyphae and the spores may be scanty. However, it was usually the finding of clumps of spores that pointed to the presence of hyphae nearby and enabled the diagnosis to be made.

It was not hard to distinguish between the septate, branching hyphae of a dermatophyte, the short mycelial elements and clumps of round spores of *Malassezia furfur*, and the thin, often sparse pseudohyphae and scattered blastospores of candida. *M. furfur* was the easiest to find and candida the most difficult. Regarding dermatophytes, the mycelium of *Epidermophyton floccosum* was usually the most prolific and easily seen with its prominent, beaded hyphae. The long, delicate hyphae of *Trichophyton mentagrophytes* were found fairly readily. The broad, more irregular hyphae of *T. rubrum* were generally short and sparse and more difficult to spot. The somewhat similar hyphae of *Microsporon canis*, although longer, were only found after a more tedious search. However, the growth of all these dermatophytes was very dense in patients treated with the prolonged application of topical steroids and the mycelium was often found at first glance (Fig. 1). Some of these cases corresponded clinically to those described as 'tinea incognito' (Ive & Marks, 1968). In the one case of tinea nigra seen, sufficient stratum corneum was stripped off the palm to allow the ready identification of the broad, dematiaceous hyphae of *Cladosporium werneckii*.

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* Acheson Colloids Ltd, Prince Rock, Plymouth, U.K.

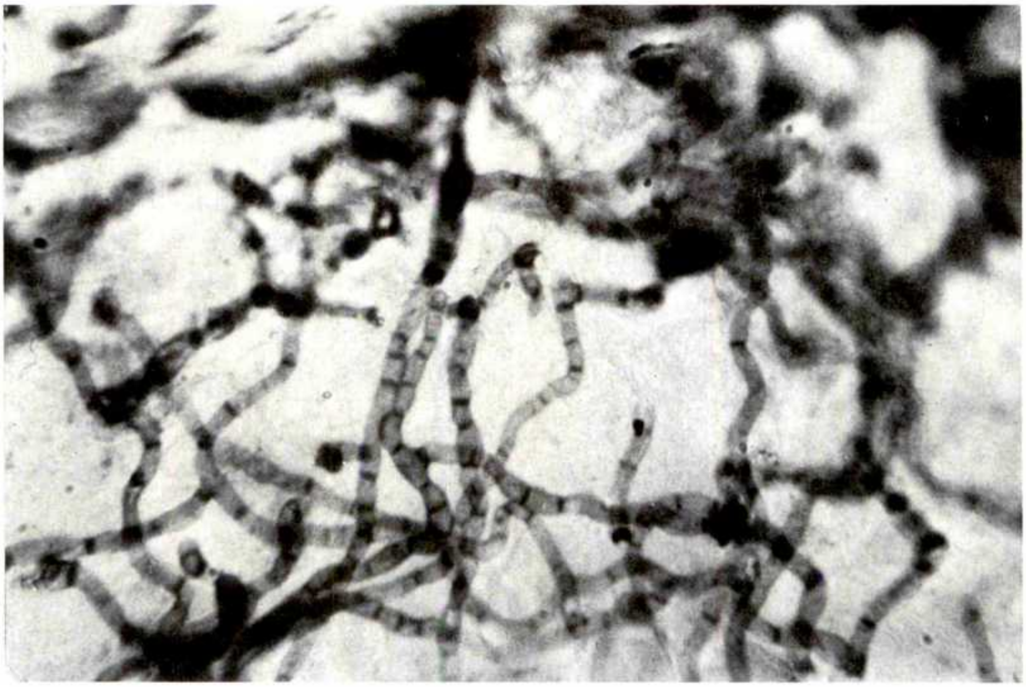

FIGURE 1. Direct examination of skin surface biopsy from a case of tinea cruris treated with prolonged topical corticosteroids shows profuse mycelium of *E. floccosum* (KOH and ink, ×360).

TABLE 2. Direct examination with PAS stain

Condition	No. of cases	Positive results
Tinea corporis	6	6
Tinea cruris	7	3
Tinea capitis	3	2
Pityriasis versicolor	8	7
Candidiasis	3	1
Erythrasma	2	2
Tinea nigra	1	1
Totals	30	22

As a further diagnostic aid, specimens taken from the scalp could, if necessary, first be examined under Wood's light, with the biopsy slide placed face downwards on the lamp. In *Microsporum canis* infections, where fluorescence was not observed on the patient due to breaking away of affected hairs, the biopsy specimen often showed marked green fluorescence around the plucked affected roots.

The PAS stain

This procedure yields permanent specimens suitable for more detailed study. The PAS-positive fungal structures stand out clearly and are easily recognized.

PAS stains were done on skin surface biopsies from thirty of the 124 patients and positive results obtained in twenty-two (Table 2). It should be noted that four cases early in the trial had negative results on PAS staining but were positive on direct examination with KOH and ink. This was probably due to a faulty staining technique which was rectified later. Another possible source of discrepancy between the PAS stain and the KOH and ink results, or likewise culture and SEM findings, is the fact that the biopsies were taken from different areas, not all of which may have been actively

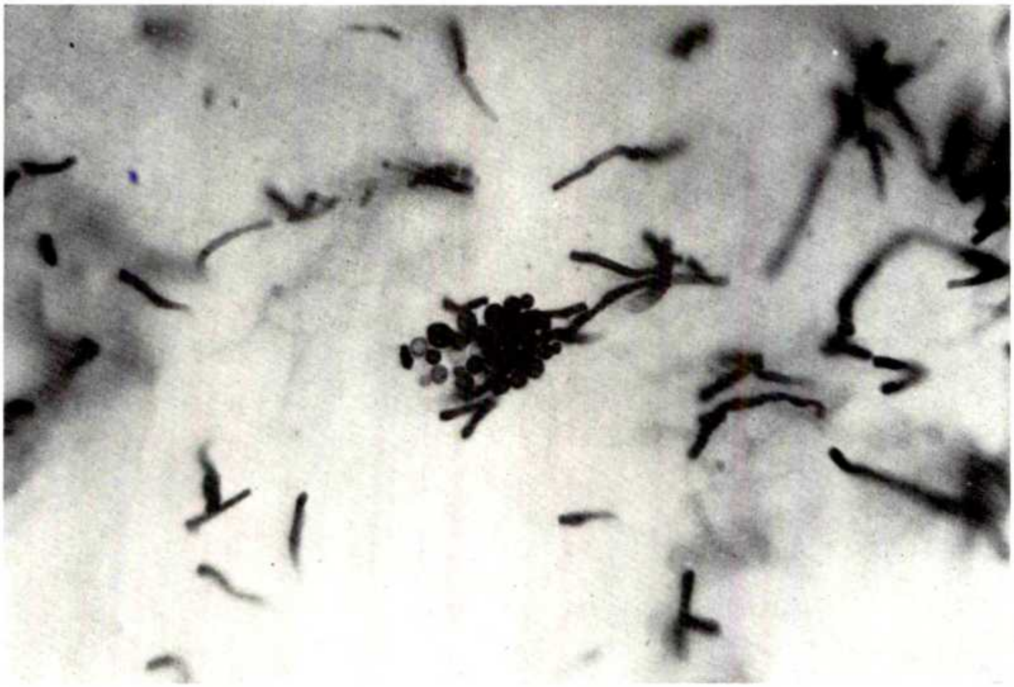

FIGURE 2. Clumped spores and mycelial elements of pityriasis versicolor are shown in this skin surface biopsy (PAS, × 360).

TABLE 3. Fungal cultures

Condition	No. of cases	Positive results
Tinea corporis	6	6
Tinea cruris	7	3
Tinea capitis	3	2
Pityriasis versicolor	7	0
Candidiasis	10	8
Erythrasma	2	2
Tinea nigra	1	1
Totals	36	22

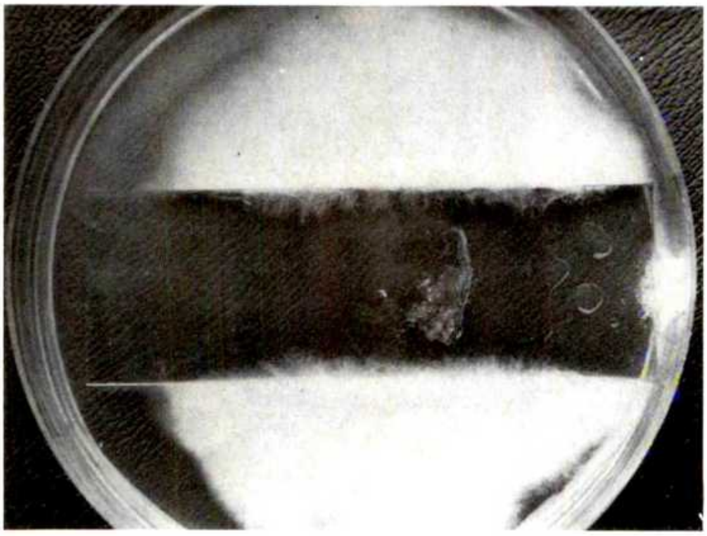

FIGURE 3. Skin surface biopsy plated on Actidione agar shows aerial mycelium of *Microsporum canis* growing outwards from the edges of the slide.

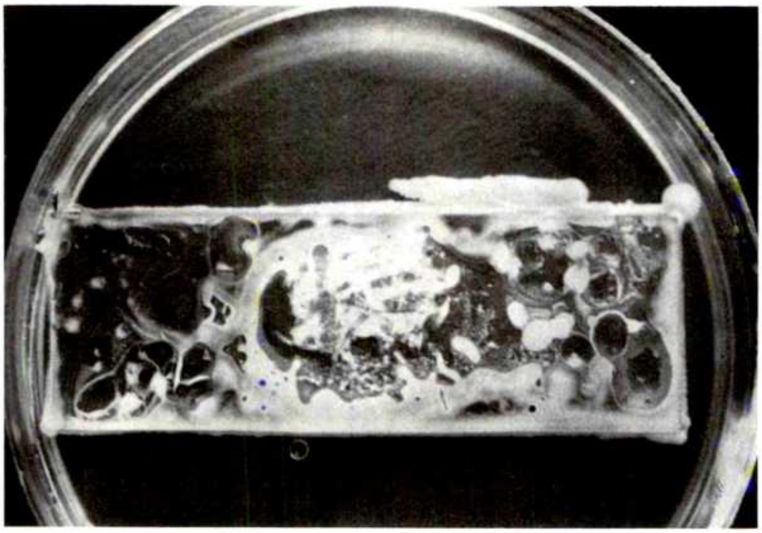

FIGURE 4. Skin surface biopsy plated on Actidione agar shows mycelium of *Candida albicans* growing under and around the slide.

infected. After allowance for these sources of error, it is evident that the PAS stain is a reliable method for the direct diagnosis of a tinea infection or pityriasis versicolor (Fig. 2). It appeared to be useful for the diagnosis of erythrasma, and this was confirmed in other cases seen after the trial period under discussion. It may, like the KOH and ink method, be less reliable for cases of candidiasis in which pseudohyphae are often hard to find and culture is still the method most likely to yield positive results.

Fungal culture

Others have shown that vinyl plastic adhesive tape strippings are suitable for fungal culture (Keddie, Orr & Liebes, 1961; Knudsen, 1971; Barnetson & Milne, 1973); skin surface biopsies were used here for a similar purpose. Specimens taken from thirty-six patients yielded positive cultures in twenty-two cases (Table 3). If cases of pityriasis versicolor are excluded, it can be seen that dermatophytes and candida species were grown from about 75% of cases. Colonies of *Corynebacterium minutissimum* were obtained easily after the organism had been found with the PAS stain, and brain–heart infusion agar substituted for the Actidione agar medium.

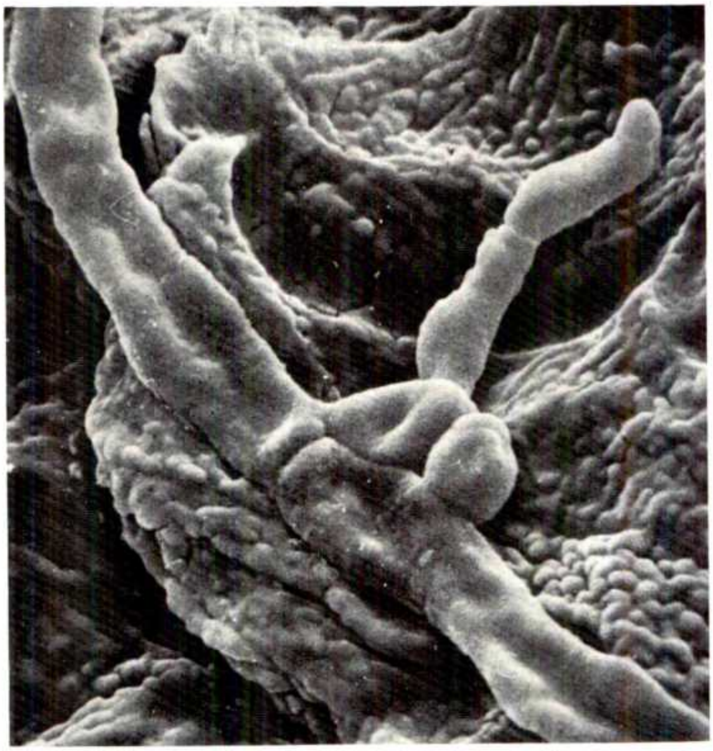

FIGURE 5. Scanning electron micrograph of skin surface biopsy from *Candida albicans* lesion showing pseudohyphae with blastospore formation (× 5000).

Fungal or bacterial growth was not impaired by the cyanoacrylate adhesive itself, possibly because of its rapid polymerization on the outer surface of the biopsy specimen. The exclusion of oxygen by the overlying glass slide did not prevent fungal growth, although it sometimes delayed it. Cultures were obtained both from the first, or 'replica' plate (Barnetson & Milne, 1973) and from the second plate. The aerial mycelium of the dermatophytes spread outwards over the plate after its initial appearance at the edges of the slide (Fig. 3), as did that of *C. minutissimum*. Candida species grew readily both under and around the glass slide (Fig. 4).

Subsequent to this trial, a positive culture of *Sporotrichum schenckii* was obtained from a skin surface biopsy specimen taken from an epidermal lesion of sporotrichosis.

Scanning electron microscopy

The preparation of skin surface biopsies for SEM was not difficult. A silver coating was found to resist disintegration better than gold, and it had to be applied more thickly to hair-bearing specimens. Surface biopsies from patients with tinea capitis, corporis or cruris, and candidiasis (Fig. 5), pityriasis versicolor and erythrasma (Fig. 6) were examined.

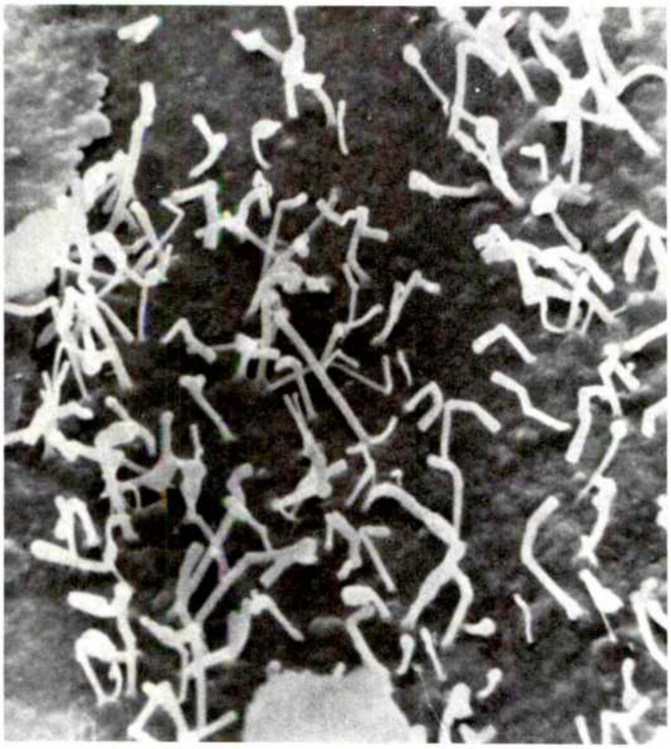

FIGURE 6. Scanning electron micrograph of skin surface biopsy from erythrasma lesion showing the angulated and clubbed organisms of *Corynebacterium minutissimum* (× 2000).

CONCLUSIONS

The skin surface biopsy method is most useful for the diagnosis and study of superficial fungal infections involving the scalp, face, trunk, groins and limbs. In a busy clinic it is a quick and easy way of collecting intact sheets of stratum corneum, and hair, which remain attached to the glass slide and arrive in the laboratory intact. A clinician, with experience in the method, can frequently diagnose the type of fungal infection by direct examination of a specimen treated with KOH and ink in the consulting room. The laboratory routinely requires two specimens, one for direct examination after PAS staining, and the other for culture.

The method has seemed so satisfactory that all hospitals in the group have been supplied with the cyanoacrylate cement for routine dermatological use, and with plastic Coplin jars for transit of specimens. A survey of the incidence of superficial mycoses in the Southern Transvaal is now under way using the facilities of one central laboratory.

ACKNOWLEDGMENTS

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Onychomycosis in elderly chiropody patients

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SUMMARY

The thickened toe-nails of 168 patients attending a chiropody clinic for old age pensioners were examined for onychomycosis by a suction drill sampling method. The nails of sixty-eight (41%) of the patients were microscopically positive. Cultures from 12% of these were unsuccessful. Of the remainder, twenty (12%) were infected by dermatophytes and 42 (25%) by moulds. In addition, large quantities of several yeast species were grown from thirteen microscopically negative nails. A higher proportion of men than of women in the same age range were infected both by dermatophytes and moulds.

There is no reliable assessment of the incidence in onychomycosis of the many fungal species associated with the condition. This is because, for reasons discussed by Gentles (1971), the success rate of cultures from microscopically positive nail clippings is usually only about 50%. Zaias, Oertel & Elliott (1969), by grinding nail clippings to a powder, obtained cultures of dermatophytes, moulds and yeasts from 93·7% of microscopically positive specimens. However, they gave no indication as to whether the last two groups of fungi were isolated in significant quantities in given cases. This is an important omission, as these organisms can often be contaminants, and their inclusion as the cause of true infections, when isolated only in small quantities, could lead to an artificially high figure for successful cultures.

English & Atkinson (1973) found that by using a suction drill to obtain specimens from the whole nail surface they could attain a culture success rate of approximately 90% while using stringent criteria for including mould and yeast isolations as genuine infections. The suction drill has been used again here to determine the incidence of onychomycosis, and of the fungi associated with it, in the toe-nails of an unselected group of elderly chiropody patients. Chiropodal work was carried out by R.A. and laboratory studies by M.P.E.

PATIENTS AND METHODS

All patients attending chiropody clinics for pensioners at Fishponds Health Centre, Bristol, from 9 to 31 October and from 6 to 22 November 1972, were included. The majority of the patients were from the lower-middle and working classes, but persons in higher social categories also attended. The women were 60 years old and over, while the men were all 65 years old or more. Women greatly outnumbered men in the '65 years and over' age group.

The drill was used only on thickened toe nails. If more than one of the patient's nails were thickened the dust from all affected nails was pooled. Most patients came for treatment of corns and callus, and to have their nails cut because they were unable to reach them themselves; only a few of those with thickened nails were complaining of them. Nails of normal thickness are very unlikely to be infected by fungi and have been considered as mycologically negative for the purposes of this survey.

The apparatus and techniques used in the clinic and laboratory were those described by English & Atkinson (1973). The same difficulties in interpretating results of culture for fungi other than dermatophytes were encountered as were described in that article, and the same criteria were used in resolving them. The bags containing the nail dusts were posted, together with particulars of the patients, to the aboratory on the day on which the patients were seen.

TABLE 1. Characteristics of total population of patients

Sex	Age group (years)	No. of patients: With thickened toe-nails	No. of patients: Total	% of patients with thickened toe-nails
F	60–64	16	22	73
F	65 +	106	141	75
M	65 +	46	53	87
Total patients		168	216	78

TABLE 2. Incidence of microscopically positive toe-nails in patients of 65 years and over

Sex	Total patients	No. with thickened toe-nails	No. with microscopically positive toe-nails	Microscopically positive: % of total	Microscopically positive: % of thickened toe-nails
F	141	106	36	26	34
M	53	46	25	47	54
Total	194	152	61	32	40

RESULTS

Table 1 shows the categories of patients and the numbers in each category with thickened toe-nails, i.e. the number from whom a sample of nail dust was obtained. Table 2 shows the relative incidence of microscopically positive toe-nails in men and women of 65 years and older; that is, it excludes women of under 65 years, as there were no men of the same age for comparison. Nearly half the men were infected but only one-quarter of the women. Hyphae could be seen in 40% of all thickened toe-nails in this age group. Cultures failed in 12% of the microscopically positive nails (Table 4).

Table 3 sets out the numbers of dermatophytes, moulds and yeasts isolated from patients aged 65 years and older. Hyphae were always visible on direct microscopy of the nails which yielded dermatophytes or moulds; yeasts were never seen on direct microscopy of the nails, even though they were isolated in very large quantities in the cases recorded. Twice as many patients were infected by non-dermatophytes as by dermatophytes. In microscopically positive nails, the percentage of the two groups of fungi was the same in both sexes; but if infections were expressed as the percentage of patients with thickened nails, or of all patients, approximately twice as many men as women were infected by dermatophytes, by moulds and by all filamentous fungi. About the same proportion of males and females harboured large quantities of yeasts.

TABLE 3. Types of fungi isolated from the toe-nails of patients aged 65 years and over

Sex	Total patients	No. of patients: From whom fungi isolated	No. of patients: With microscopically positive nails	%	No. of patients with thickened nails	% from whom fungi isolated	% of total patients from whom fungi isolated
(a) Dermatophytes (microscopically positive)							
F	141	10	36	28	106	9	7
M	53	7	25	28	46	15	13
Total	194	17	61	28	152	11	9
(b) Moulds (microscopically positive)							
F	141	21	36	58	106	20	15
M	53	15	25	60	46	33	28
Total	194	36	61	58	152	24	17
(c) Yeasts (microscopically negative)							
F	141	10	—	—	106	9	7
M	53	3	—	—	46	7	6
Total	194	13	—	—	152	9	7

The nails of many patients, whether or not they were positive on direct microscopy, yielded a few colonies of assorted moulds and yeasts in culture. As in our previous study, these were not considered to be of clinical significance and are not recorded here.

Table 4 shows the number of each species of fungus isolated from the whole group of patients (including the sixteen women under 65 years old) and considered to be of clinical significance on the basis of the criteria laid down in our previous study. The footnote details the mixed infections. *Trichophyton interdigitale* was much the most common dermatophyte isolated, but of the sixteen nail infections by this fungus, only seven had associated skin lesions. All four *T. rubrum* and *Epidermophyton floccosum* cases had skin lesions due to the same fungi.

With two exceptions, all the moulds isolated have been reported from nails before. *Pyrenochaeta* sp. has not been recorded from this site previously. It is a pycnidial fungus, that is, one in which the minute, colourless, asexual spores develop within a dark-coloured, thick-walled receptacle known as a pycnidium. When the spores are ready for liberation they are extruded in a slimy mass through a small opening in the wall of the pycnidium. One of the *Acremonium* (*Cephalosporium*) isolates is an undescribed species (Hawksworth, personal communication). Among the yeasts, neither *Candida mogii* nor *Torulopsis haemulonii* has been found in nails previously.

TABLE 4. Species of fungi isolated from all the patients examined*

Fungus	No. of cases	% of total dermatophytes
Dermatophytes		
T. interdigitale	16	80
T. rubrum	2	10
E. floccosum	2	10
Total	20	
		% of total non-dermatophytes
Moulds		
Scopulariopsis brevicaulis	3	7
Aspergillus terreus	16	38
A. versicolor	12	29
A. nidulans	1	2
A. candidus	1	2
Acremonium (*Cephalosporium*) spp.	8	19
Pyrenochaeta sp.	1	2
Total	42	
Yeasts		
Candida parapsilosis	3	
C. guilliermondii	1	
C. mogii	4	
Torulopsis haemulonii	3	
Rhodotorula rubra	2	
Total	13	

Failed cultures

No. failed	No. microsc. positive	% failed
8	68	12

* Fungi from double infections are listed separately in the Table. They included one case each of:

T. interdigitale + *E. floccosum*
S. brevicaulis + *A. versicolor*
A. terreus + *C. parapsilosis*
A. versicolor + *A. nidulans*

DISCUSSION

There is no reason to believe that the 216 patients seen in this survey are in any way unrepresentative of those attending chiropody clinics for pensioners, yet the incidence of thickened toe nails is extraordinarily high. Walshe & English (1966) have shown that the incidence of abnormal toe-nails increases with age whether or not the nails contain a fungus. In the present study, the underlying causes of the thickening were not investigated, but could probably be attributed to fungi in the cases of *T. rubrum*, *E. floccosum*, *Scopulariopsis brevicaulis* and some *T. interdigitale* infections. In those *T. interdigitale* infections which were largely superficial and which were not accompanied by skin lesions, and in all mould infections except those due to *S. brevicaulis*, it is more than likely that the nails were already dystrophic when colonized saprophytically by fungi (English & Walshe, 1970; Schönborn & Schmoranzer, 1970). The toe-nails of elderly people, because they are so frequently abnormal, would provide an especially favourable substrate for the growth of saprophytic fungi. This would account for the fact that in this study 18·5% of all patients seen, and 68% of all those with positive microscopy and from whom fungi were isolated, were infected by moulds. These results support those of Schönborn & Schmoranzer (1970) and Liautaud, Grosshans & Bassett (1971), who found that a large proportion of their patients were elderly.

In clinical practice it may be important to distinguish between dermatophytes and moulds in microscopically positive nails, as treatment with griseofulvin would be of use only against dermatophytes. The high rate of cultural failure by normal sampling methods often makes this distinction impossible. The demonstration in this study of the very large proportion of cases of onychomycosis of the toe-nails which are attributable to moulds emphasizes that some alternative means of distinction would be valuable when cultures are unobtainable. As it is extremely rare for a mould to cause skin lesions, a clinical picture of microscopically positive nails, either without skin lesions, or with lesions of non-mycotic origin, would give a reasonably accurate diagnosis of onychomycosis due to a mould. Some *T. interdigitale* infections without accompanying skin lesions would unavoidably be included with the moulds by such a deductive method; but *T. rubrum* and *E. floccosum* nail infections would be clearly separated from them.

The great preponderance of *T. interdigitale* over the other dermatophytes is in agreement with the results of Walshe & English (1966) and with our own previous experience (English & Atkinson, 1973). It confirms the fact that in Britain, in groups of persons of whom only a small proportion are complaining of their toe-nails, *T. interdigitale* is much more common than *T. rubrum*. Walshe & English also noted a high proportion of *T. interdigitale* toe-nail infections without associated skin lesions.

It has long been recognized that tinea pedis due to *T. interdigitale* is more common in males than in females; our results show that this is also true of tinea unguium due to this fungus, at least in elderly people. In onychomycosis due to moulds, Liautaud *et al.* (1971) and Schönborn & Schmoranzer (1970) reported that more women than men were infected. But these are actual numbers, not percentages, and neither group of workers indicated the total number of each sex in the population examined. Consequently their results may not necessarily be at variance with ours, in which many more women than men were seen, but which showed that in persons of 65 years and over, onychomycosis due to moulds is more common in men (33%) than in women (25%) with abnormal toe-nails. The difference is even greater (28% men to 15% women) if the percentage is calculated on the whole population examined (Table 3). This difference could be partially, but not entirely, accounted for by the fact that 10% more men than women have thickened toe-nails and that more of them are therefore vulnerable to infection.

Though a greater variety of yeasts was isolated on this occasion than in our first attempt at drill sampling, no further clues were obtained as to their significance. That *C. mogii* and *T. haemulonii*, recorded for the first time, should have been isolated four and three times respectively is perhaps less surprising than it appears. Both yeasts are very tolerant of high salt concentrations (about 20% NaCl) and this osmophilia could well account for their ability to colonize such an apparently inhospitable substrate as a toe-nail. That they have never been noted in this site before could be due to the novel sampling method and the fact that, as does not always happen in diagnostic work, a considerable effort was made to identify them.

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We are grateful to Mr J.Pugh, Chief Chiropody Officer, City and County of Bristol, for his encouragement of this study; to the Commonwealth Mycological Institute for identification of two of the moulds; to Dr M.Smith, of the Centraalbureau voor Schimmelcultures, Delft, Holland, for the identification of *C. mogii* and *T. haemulonii*; and to Mrs L.Lewis for technical assistance.

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Glucose tolerance in lichen planus

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SUMMARY

Glucose tolerance has been investigated in twenty-one patients with lichen planus. 62% of these patients have shown abnormal results, suggesting impaired tolerance.

The aetiology of lichen planus remains unknown. Evidence has been found for an infectious cause but this hypothesis remains unproven. More recently, the metabolic function of the affected epidermal cell has been investigated with histochemical and biochemical techniques, revealing abnormalities of enzyme activity (Cotton, van der Hurk & van der Staak, 1972). Jolly (1972) proposed that a generalized metabolic defect might be associated with lichen planus, and he demonstrated abnormal glucose tolerance, suggesting impaired carbohydrate metabolism, in patients with lichen planus. This investigation is an attempt to verify these findings; the provisional results are now available.

METHOD

Twenty-one patients with lichen planus, attending dermatology out-patient clinics, have been investigated. All patients showed classical, clinical and/or histological evidence of this condition; in most, both the skin and the oral mucous membrane were involved. Patients were asked particularly about any personal or family history of diabetes mellitus. All patients except those known to be diabetic were then investigated by means of a standard 50 g oral glucose tolerance test. Capillary blood specimens were taken fasting, and at half-hour intervals after the administration of glucose, for 2½ h. Glucose levels were estimated by the glucose oxidase method. The results of the glucose tolerance tests were interpreted as being abnormal when the fasting blood sugar was above 100 mg/100 ml, when there was a high peak (>170 mg/100 ml for those under 45 years and >200 mg/100 ml for those over 45), or when the 2 h level was raised (160 mg/100 ml being definitely abnormal and 120–160 mg/100 ml suspicious) (Mahler & Hayes, 1973). No patient was receiving systemic corticosteroids at the time of investigation, although some were applying small amounts of corticosteroid to the skin.

RESULTS

The age and sex distribution is shown in Table 1. When compared with the age distribution for all patients with lichen planus seen over a 12-month period, the pattern is similar.

There were six patients aged under 45. Two of these had normal glucose tolerance and one was a known diabetic. Three had abnormal results; two, peak levels above 170 mg/100 ml, and one a 2 h level above 120 mg/100 ml (Table 2). In two of these, diabetes mellitus had occurred in the family.

TABLE 1. Patients investigated: number by age and sex

Age (years)	Male	Female	Total
< 30	2	2	4
30–39	1	1	2
40–49	0	2	2
50–59	3	3	6
60–69	1	1	2
70–79	4	1	5
Total	11	10	21

TABLE 2. Patients under 45 years

No. of patients	Normal	Known diabetes mellitus	Abnormal results			Family history of diabetes mellitus
			Fasting	Peak	2 h	
1		Yes				Yes
1					120	Yes
1				195		
1				185		Yes
1	Yes					Yes
1	Yes					
Total: 6	2	1	0	2	1	4

TABLE 3. Patients over 45 years

No. of patients	Normal	Known diabetes mellitus	Abnormal results			Family history of diabetes mellitus
			Fasting	Peak	2 h	
5	Yes					
1	Yes					Yes
1		Yes				
1			105			
1			105	225		Yes
1			100	225		Yes
1					125	
1					135	Yes
1					140	
1			110	215	120	
1			125	245	210	Yes
Total: 15	2	1	5	4	5	5

Fifteen patients seen were aged over 45 (Table 3). Six had normal glucose tolerance and of these only one had a family history of diabetes mellitus. One was a known diabetic. Two showed abnormal results for the fasting, peak, and 2 h levels; one of these has a sister who is a diabetic. Three patients had 2 h levels between 120 and 160 mg/100 ml, and one of these gave a family history of diabetes mellitus. Two patients, each with a positive family history, had raised fasting levels and raised peak levels and one patient had a raised fasting level.

Taking all patients together, of twenty-one investigated, thirteen (62%) showed some abnormality.

DISCUSSION

Abnormal glucose tolerance in 62% of the subjects studied is a high figure. Results of the Bedford survey showed that 12–14% of the adult population may have an abnormal glucose tolerance test. This figure is based on a 2 h blood glucose of over 120 mg/100 ml (Butterfield, 1964). A diabetes survey undertaken in Birmingham by the College of General Practitioners (1962) showed abnormal glucose tolerance in twelve out of 123 control patients. It also demonstrated an increase in the incidence of abnormal findings with increasing age, but the criteria for abnormality were not age related.

As far as our abnormal findings are concerned, it is true that not all the patients satisfy all criteria (with regard to the glucose tolerance test) for the diagnosis of diabetes mellitus. These are satisfied in four patients (19%).

The most striking abnormality in glucose tolerance is the large number showing high peak levels. In addition to those shown in the tables, several of the patients aged over 45, in whom the glucose tolerance was classified as normal, had peak levels of 170–200 mg/100 ml.

Further investigation is in progress to verify these results and to provide information about glucose tolerance in a larger group of patients with lichen planus.

ACKNOWLEDGMENT

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Differential diagnosis of ischaemic ulceration of the leg using ultrasound

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SUMMARY

Whilst most ulcers on the leg can be confidently and easily separated into those due to venous insufficiency and those due to arterial obstruction, some may present problems of differential diagnosis. Further investigation then becomes necessary. In order to avoid arteriography a new, quick, transcutaneous method has been evolved, which is painless, repeatable and can be carried out in an outpatient clinic. This technique uses two ultrasonic flowmeters. The propagation time of the pulse wave between two measuring sites along the arterial system of the leg and the change in waveform shape between these two sites are measured.

METHOD

It has been shown previously (Woodcock, 1970; Fitzgerald, Gosling & Woodcock, 1971; Woodcock, Gosling & Fitzgerald, 1972) that the measurement of pulse transit time between two sites along the arterial system of the leg, and the change in shape of the blood-velocity/time waveform between these two sites can be used to classify the collateral circulation present in cases of complete occlusion. It is also possible to distinguish between various conditions of atheromatous involvement in partial stenosis, and to give an overall assessment of 'limb viability'.

The rationale of this technique is to exclude the arterial system as the major contributing factor in ischaemia, thus implicating the venous system. The details of the method are exactly the same as those described elsewhere (Woodcock, 1970; Fitzgerald *et al.*, 1971; Woodcock *et al.*, 1972). The arterial system of the leg is assessed by placing an ultrasonic flow-velocity meter over the common femoral artery and a second one over the popliteal artery in the popliteal fossa. The flow-velocity meter emits, through the skin and underlying tissue, a beam of ultrasound of frequency 10 MHz, which is directed towards the blood vessel. The incident energy is scattered by the moving blood cells and the scattered wave has a frequency different to that of the emitted wave (Doppler effect). In the physiological range of blood velocity, the Doppler shift frequencies, which are directly proportional to the velocity of the blood, are in the audio frequency range and can be recorded by a standard tape recorder. By using two Doppler shift flow-velocity instruments simultaneously at the femoral and popliteal, and at the popliteal and posterior tibial arteries, it is possible to measure two important parameters; (1) the transit time of the pressure wave caused by contraction of the left ventricle, this transit time being related to the thickness of the blood vessel wall, the lumen radius and the vessel wall elasticity, and (2) the blood velocity at each site over the cardiac cycle. For convenience these simultaneously measured signals are recorded by a tape recorder and analysed when required.

There are two principal ways of analysing the Doppler shift signals. First, a zero crossing frequency meter which gives a weighted average of all the Doppler shift frequencies present in the scattered signal, and secondly, audio frequency analysis which shows all the frequencies present. The choice of method depends upon factors which have been discussed elsewhere (Gosling *et al.*, 1969) but can be summarized as follows: the zero crossing frequency meter is very sensitive to the presence of noise and cannot resolve two signals, for example from an artery and its associated vein; the audio frequency analyser displays all signals and can distinguish between arterial and venous flow.

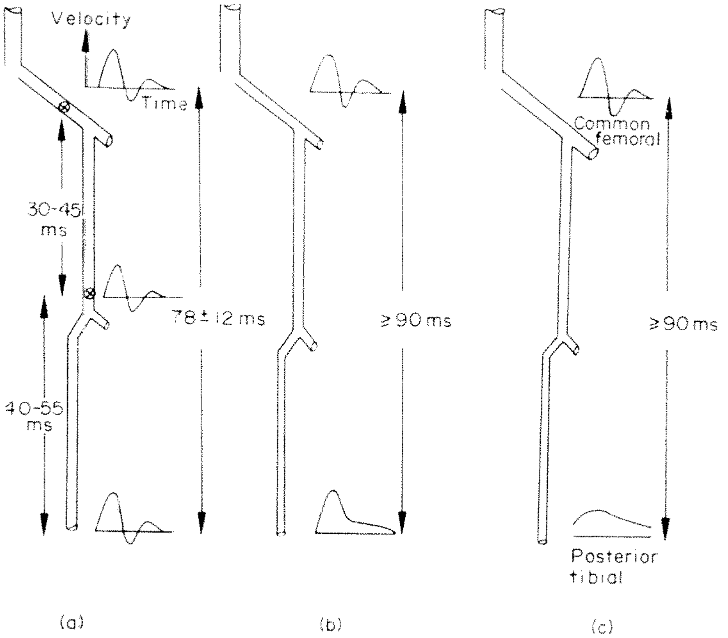

FIGURE 1. Transit times and blood-velocity/time waveform shapes at and between the common femoral and posterior tibial arteries in man. If the measurements are as shown in (a), then there is no arterial disease and the venous system is implicated; if as in (b) or (c), then the arterial supply will be reduced.

In measuring the change in shape of the blood-velocity/time waveform, the output of the zero crossing frequency meter or the maximum frequency envelope over the cardiac cycle is used. This change in shape can be quantified using Fourier analysis, or for a quick appraisal can be viewed by eye. The variation of the blood velocity over the cardiac cycle in normal and in atherosclerotic arteries is shown in Fig. 1. It can be seen that when a complete block of the femoral artery is present then a longer transit time is introduced, and the shape of the blood-velocity/time waveform changes from being completely pulsatile to one where there is flow over the whole cardiac cycle and the waveform shape is damped. Also shown in Fig. 1 are typical waveform shapes and transit times for cases of atheromatous involvement in which there is an incomplete block. If the results from the patient analysis resemble 1(a), the arterial system is free from major vessel disease and the cause of the ulceration is probably poor venous drainage. If the results, however, resemble 1(b) and (c), then the arterial system could be implicated and the proposed treatment should be modified in this light.

RESULTS

Seven patients were investigated in an initial trial and the results are summarized in Table 1.

L., female, aged 47, had had a stasis ulcer on the left external malleolus for 10 years; it healed with pressure bandaging but recurred 4 months later and healed again in 8 weeks, only to recur again and heal again.

TABLE 1.

Patient	Transit time (ms) Fem.–popl.	Transit time (ms) Popl.–post. tib.	Change in blood-velocity *v.* time waveform shape	Diagnosis: ischaemia due to
A.	35	55	Yes	Poor arterial supply
L.	40	30	No	Poor venous drainage
E.	35	40	No	Poor venous drainage
B.	40	35	No	Poor venous drainage
F.	30	30	No	Poor venous drainage
H.O.	35	30	No	Poor venous drainage
H.	75	75	Yes	Poor arterial supply

B., female, aged 60, was an obese patient with a stasis ulcer on the medial aspect of the left ankle, and varicose veins along the course of the saphenous system with incompetent perforators over both legs. Ulcer healed with injection and compression therapy.

E., female, aged 63. Many stasis ulcers. Healed with compression therapy.

A., male, aged 65. Atrophie blanche, ulcer (traumatic) and stasis contact eczema.

F., male, aged 82. Many stasis ulcers, healed with compression therapy.

H., male, aged 59. Many small ulcers, did not respond to compression therapy.

H.O., female, aged 46. Large stasis ulcer on medial aspect of right ankle.

Of the seven patients examined, five were considered to have venous impairment, and in two the arterial supply was implicated in the ischaemic ulceration. The five who were considered to have poor venous drainage were treated conservatively with local applications and compression therapy, and made good progress towards complete recovery. Patient H.O., although found to be suffering from venous impairment, complained of pain in the calf of the affected leg after exercise. An arteriogram was performed which confirmed the absence of any arterial disease. An arteriogram was performed also on patient H., which confirmed a complete block in the superficial femoral artery, and further gross involvement of the vessels distal to the popliteal trifurcation.

The results in two cases, patients A. and L., are described in detail below, and summarized in Fig. 2. Patient A. has transit times and changes in the blood-velocity/time waveform consistent with major localized plaques in the posterior tibial artery. The transit time between the right common femoral and right popliteal arteries was 35 ms, which is within the normal range of 30–45 ms, and the blood-velocity/time waveform at the popliteal artery is normal. In the popliteal–posterior tibial segment the transit time lies within the normal range, 40–55 ms, but the posterior tibial waveform is severely damped. This suggests major localized atheromatous plaques in the posterior tibial artery. If the transit time in this segment was faster than normal, then the vessel would be narrowed over most of its length, but since the transit time is unaffected the damping must be caused by localized plaques.

In the second patient, L., both the transit times were within the normal range, and the blood-velocity/time waveform shapes were all normal (see Fig. 2). Thus there is no evidence of any arterial disease, suggesting that the ischaemia is of venous origin.

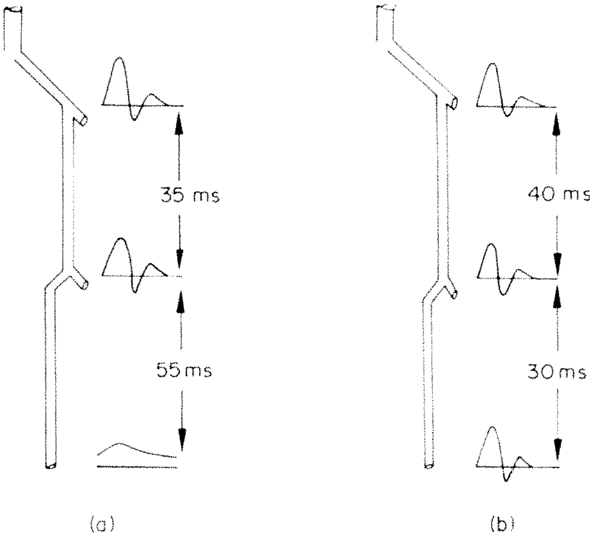

FIGURE 2. Transit times and blood-velocity/time waveform shapes recorded in two patients. Case (a), patient A., shows signs of arterial disease; case (b), patient L., shows no signs of arterial disease.

DISCUSSION

The technique of transit time and blood-velocity/time measurements can be used to diagnose arterial disease and provides a useful method for distinguishing ischaemia due to poor arterial supply from that due to poor venous drainage. The method described is transcutaneous, quick and painless, and can be repeated as often as necessary. In two of the seven patients investigated the diagnosis was confirmed by arteriography, and five were found to have impaired venous drainage which responded well to compression treatment. The remaining two patients had an impaired arterial supply.

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Lichen sclerosus et atrophicus in the Bantu

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SUMMARY

Four cases of lichen sclerosus et atrophicus are described. This condition has hitherto been regarded as very rare in the pure-blooded African, and these cases are believed to be the first described amongst the African Bantu population.

Lichen sclerosus et atrophicus (LSA) is an uncommon disorder first described by Hallopeau and Darier in 1887. It is characterized by the presence of small angulated lesions, off-white or ivory in colour, with a shiny translucent appearance. Lesions may become aggregated into plaques showing a typical cigarette paper-like wrinkling of the surface. Hyperkeratosis and follicular plugging may occur. The latter has been named 'delling'. Well defined erythematous zones may surround the plaques. Histological criteria for diagnosis include the presence in the upper layer of the dermis of typically hyalinized and oedematous material, with disappearance of the elastic tissue.

Atrophy in LSA is frequent, especially in the vulvar region.

LSA is usually symptomless, except in the anogenital area, where it may cause intense itching and soreness.

The frequency of LSA in Britain was reported by Wallace (1971) to vary between 1 in 1000 and 1 in 300 of dermatological patients, depending on the liaison between the skin and gynaecological departments.

The condition has always been regarded as being rare in African Negro practice. However, a number of cases have occurred in recent years in the American Negro, and Irgang (1944) claimed he described the first case in such a patient. Senear (1948) referred to a case of LSA in a 52-year-old Negro woman, and J.L.Miller (1957) discussed a case in which the typical lesions of LSA on the back co-existed with clinically and histologically confirmed scleroderma 'en bande' on the foot. Chernosky, Derbes & Burks (1957) included one Negro amongst five children affected by LSA, and stated that they had observed other Negroes with this condition. Barclay, Macey & Reed (1966) described five cases of LSA in Negro children in New Orleans.

In contrast, a search of the literature available to us failed to reveal a single case of LSA in the African Negro. Findlay (1966) wrote that no case of LSA had been diagnosed in a Bantu in his department at Pretoria. Scott (1972) stated it to be very uncommon amongst the Bantu of the Orange Free State, and no case has been seen amongst the Bantu attending the skin department at the University of Natal (Bentley-Phillips, 1973). Clarke (1959) claimed to have seen this skin disease in Nigeria, but gave no details.

We therefore describe what are believed to be the first documented cases of LSA in the African Bantu.

CASE REPORTS

Case 1

A 19-year-old schoolgirl had for 3 months noticed a small, hard, slowly growing, thickened pruritic lesion below the right ear. This was an area, 5 cm × 3 cm, of indurated atrophic skin, hyperpigmented at the centre and hypopigmented at the periphery. There was also a symptomless white elevated plaque-like lesion on the edge of the tongue. Histological examination showed moderate hyperkeratosis and mild colliquative degeneration of the basal layer with consequent incontinence of pigment. The upper dermis showed homogenization of the connective tissue with decreased staining properties, and there was a scattered lymphocytic infiltrate, mainly in peri-adnexal areas (Fig. 1).

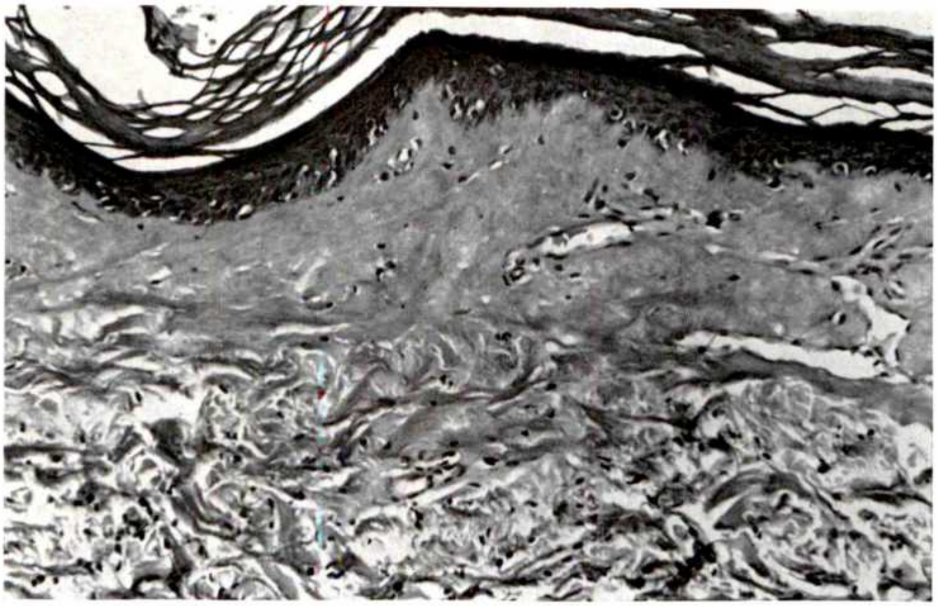

FIGURE 1. Case 1. LSA. Hyperkeratosis with some atrophy of the Malpighian layer, and well marked homogenization of the upper dermal collagen (H & E, × 120).

Case 2

An 8-year-old girl had recently developed a symptomless rash around the genitalia. The plaque-like lesion on the vulva and around the anus had a figure-of-eight distribution (Fig. 2). The labia majora and minora and the clitoris showed a waxy pallor, mild erythema and early atrophy. The clinical appearance was typical of LSA, but histological confirmation was not possible as consent for biopsy was refused.

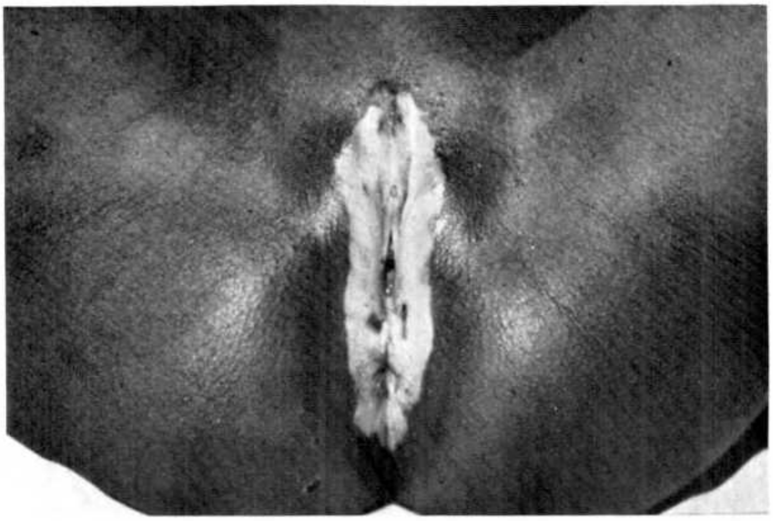

FIGURE 2. Case 2. Anogenital LSA in an 8-year-old African Bantu.

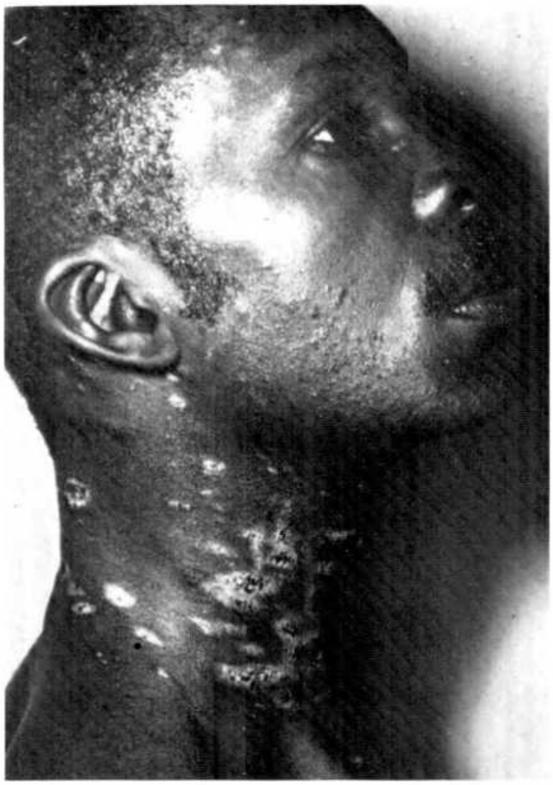

FIGURE 3. Case 3. LSA lesions in a 43-year-old African Bantu male.

Case 3

A 43-year-old clerk had for some months suffered from pruritic lesions on the neck. He was found to have a number of plaques and papules on both sides of the neck and on the forehead. Many of these were white and atrophic and showed typical cigarette paper-like wrinkling of their surface. Follicular plugging, hyperkeratosis and central hyperpigmentation were evident (Fig. 3). Biopsy showed hyperkeratosis, prominence of the granular layer, atrophic changes in the epidermis, hyalinization of the upper dermis, a mild chronic inflammatory infiltrate in the mid and upper dermis, and incontinence of pigment.

Case 4

A 21-year-old female teacher had noticed a symptomless rash for a month. She had large atrophic plaques and small white papules, scattered around the entire perimeter of the neck. Both hyper- and hypo-pigmentation were present. Biopsy showed hyperkeratosis and atrophy of the epidermis. The dermis was composed mainly of dense hyalinized collagenous tissue in the upper portion, while an increase in the collagen of the deeper dermis encroached on the sweat glands. A round cell infiltrate was present in the dermis, mainly perivascularly. It was felt that these features were more in keeping with a diagnosis of LSA than of circumscribed scleroderma.

DISCUSSION

On clinical and histological grounds, cases 1, 3, and 4 were consistent with a diagnosis of LSA. Case 2 should also be included on clinical grounds, although it was not proven by biopsy.

Case 1, though clinically and histologically LSA, was unusual in that the tongue was involved. Ravitz & Welch (1957) described three patients with involvement of the buccal mucosa, in one of whom the lesions were confined to the tongue. R.F.Miller (1957) also noted such a case.

Case 2 occurred in a young girl. Clark & Muller (1967), reviewing the literature, discovered eighty-two cases of LSA in children; the disease had generally appeared before the age of 7 years; the anogenital region was commonly affected but 25% of patients had only extragenital involvement.

Case 3, like cases 1 and 4, shows well the existence of hyperpigmented areas in otherwise typical pale waxy lesions. This is a feature that appears to be common in Negro patients.

Case 4 was difficult to assign to a diagnosis either of LSA or of circumscribed scleroderma. Steigleder & Raab (1961) found it possible to distinguish between the two conditions by special histochemical techniques. Montgomery (1967) also believed that they were two distinct diseases, but admitted that they may merge into one another. He suggested that the obsolescent term 'White Spot Disease' might be used for such borderline cases. In circumscribed scleroderma, there is no delling, plugging or marked atrophy, but there is definite sclerosis of the dermis with obliterative vessel changes.

These four cases of LSA presented to the Skin Department of the Baragwanath Hospital within a period of 3 years, during which time more than 12,000 new patients were seen.* LSA must therefore be considered a rare disease in the African Negro, but the reason for racial variation in incidence is obscure. Multiple cases in one family have been reported (Barker & Gross, 1962; Wallace, 1971), but no clear genetic pattern can be established. Neither does there appear to be any obvious environmental factor to account for apparent racial differences. Findlay (1969) suggested differences in multifactorial predisposition in the various population groups. He related this to other differences in connective tissue behaviour, notably to changes in the cervix and in the uterine supports, which are determined by the genetic capacity of the tissue to respond.

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* Since submission of this paper, a fifth case of LSA has presented to our department. She is a 7-year-old African Bantu girl with anogenital involvement, showing a clinical picture very similar to case 2. The histological features were typical of LSA.

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Pharmacology and Treatment

The treatment of idiopathic hyperhidrosis by glycopyrronium bromide and tap water iontophoresis

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SUMMARY

A method of treating idiopathic hyperhidrosis using direct current iontophoresis is described. Employing a solution of glycopyrronium bromide, such treatment produces prolonged periods of suppression of palmar and plantar hyperhidrosis but only occasionally do useful periods of dryness result from treatment of axillary hyperhidrosis.

Tap water iontophoresis can also be used to produce sweat suppression but its effects are generally less satisfactory.

The medical treatment of hyperhidrosis is usually ineffective in all but the mildest cases. Many topical agents have been used, including aluminium chloride (Shelley, 1954), potassium permanganate, formaldehyde (Shelley, Laskas & Satonove, 1954; Papa, 1966), glutaraldehyde (Juhlin & Hansson, 1968), in addition to a large number of anticholinergic compounds (Shelley & Horvath, 1951; MacMillan, Reller & Sunder, 1964; Grice & Bettley, 1966). All have shortcomings in practice. The anticholinergic compounds, particularly, have little effect when used directly on palms and soles and when taken orally, the severity of the intestinal and ocular side effects limits their usefulness.

The surgical approach to palmar and plantar hyperhidrosis by sympathectomy is an effective procedure but has occasional complications, including Horner's syndrome and damage both to pleura and phrenic nerves (Cloward, 1969; Greenhalgh, Rosengarten & Martin, 1971). The few long term studies available suggest that the anhidrosis is usually permanent (Greenhalgh *et al.*, 1971; Ellis & Naunton Morgan, 1971). Axillary hyperhidrosis may now be effectively treated by local axillary resection (Hurley & Shelley, 1966; Gillespie & Kane, 1970; Davis, 1971).

In 1968, Levit described the use of iontophoresis of tap water for palmar and plantar hyperhidrosis. Grice, Sattar & Baker (1972) have recently published results of iontophoresis of tap water and of poldine methosulphate (Nacton).

Glyco- and hexopyrronium bromide are closely related quaternary ammonium compounds showing marked anticholinergic properties (Sun, 1962), and glycopyrronium bromide (Robinul) has been extensively used for the treatment of peptic ulceration (Feder, Hadidi & Kahn, 1963; Lampier, Soegell & Goldberg, 1962; Amure, 1965; Barman & Larson, 1963). Hexopyrronium bromide was found by Stoughton *et al.* (1964) to produce satisfactory sweat suppression when used topically and was without local or systemic side effects.

It failed, however, to show measurable suppression of palmar sweat. Shelley & Horvath (1951) also found this when using scopolamine hydrobromide which they attributed to failure of penetration. After introducing scopolamine by iontophoresis they were able to show an effect lasting 10 days. Iontophoresis of hexa- and glycopyrronium bromide was therefore undertaken in patients with moderate or severe idiopathic hyperhidrosis and the results compared with those previously obtained using tap water.

MATERIALS AND METHODS

Ionization was undertaken using a direct current apparatus similar to that described in detail by Levit (1968) and widely available in physiotherapy departments. Malleable electrodes (tin) of a size comparable to the treated part were uniformly separated from the skin surface by several layers of lint. A shallow plastic tray containing the electrode allowed the addition of tap water or of an 0·1% solution of the anticholinergic drug in distilled water to a sufficient depth to cover the palmar or plantar skin. This electrically formed the anode. For axillary treatment the lint was soaked in the solution and the electrode held in place by bandaging. The cathode, a similar electrode system, using tap water in all cases, was attached to an untreated limb. Gradually increasing current was administered to skin tolerance (15–20 mA adults, 7–12 mA children) and maintained at each site for 15 min.

Twenty-six cases (sixteen female, ten male) of average age 25 years were treated with tap water twice weekly and subsequently weekly where possible. Seven received treatment to both hands and feet concurrently. Glyco- or hexopyrronium bromide was used on twenty-seven patients (fifteen female, twelve male) of average age 25 years at intervals determined by the duration of freedom from excessive sweating.

RESULTS

Table 1 briefly indicates the results of tap water iontophoresis. After a variable number of treatments to hands the hypohidrotic effect was usually sufficient to suppress symptoms between the twice-weekly visits. Four remained dry between weekly attendance. Treatments were not continued for more than 6 months, and the length of treatment did not influence the final result. Five patients did however continue to remain symptom free for between 4 and 8 weeks before relapsing, after receiving five to

TABLE 1. Tap water iontophoresis

	No. of cases	Treatment interval	Mean no. of treatments before continuous suppression of hyperhidrosis	Case no.	Comment
Palms	21	Twice-weekly (17)	7	4	Continuous suppression of sweating not achieved
		Weekly (4)		5	Improved for up to 8 weeks on stopping treatments
Soles	9	Twice-weekly (7) Weekly (2)	9	3	Improved for up to 8 weeks on stopping treatments
Axillae	3	Twice-weekly (3)	12	1	Continuous suppression of sweating not achieved

TABLE 2. Glycopyrronium bromide iontophoresis

	Case	Sex	Age (years)	Mean duration dry per treatment (days)	No. of treatments	Comment
Palms	1	M	23	49	2	
	2	M	21	38	4	
	3	F	17	31	11	
	4	F	8	18	3	
	5	F	19	35	4	Discontinued as hyperhidrosis did not recur
	6	M	27	35	1	
	7	F	17	24	6	Abdominal discomfort
	8	F	23	28	10	
	9	F	44	35	5	
	10	F	17	28	9	
	11	F	23	42	3	Discontinued as hyperhidrosis did not recur
	12	M	20	35	1	
	13	F	27	28	5	
	14	M	40	28	6	
	15	M	21	56	6	
	16	M	38	28	3	
				Mean 33·7		
Soles	17	M	22	56	5	Discontinued as hyperhidrosis did not recur
	18	M	32	77	3	
	19	F	17	7	4	
	20	M	38	49	1	
				Mean 47·2		
Axillae	21	M	34	3	4	
	22	F	27	5	3	
	23	F	26	1	1	
	24	M	24	1	2	
	25	F	23	6	3	Abdominal discomfort
	26	F	21	14	1	
	27	F	32	21	3	
				Mean 7·3		

eighteen treatments (mean twelve). Plantar and axillary iontophoresis showed similar results but required more treatments before achieving useful dryness. Provided adequate care is taken in positioning the electrodes, no side effects result from this technique.

Table 2 sets out in detail the results of glyco- and hexopyrronium bromide iontophoresis. The two drugs produced exactly similar effects and glycopyrronium is now used exclusively as it is cheaper. It became immediately apparent that a prolonged hypohidrotic effect resulted from iontophoresis of these drugs into palmar and plantar skin. In only two cases (4 and 19) did this effect last for less than 14 days after the initial treatment, and subsequent treatments generally led to extended periods of absence of visible sweating. Two cases (15 and 18) were eventually controlled by iontophoresis at 3-monthly intervals. Three patients (cases 5, 11 and 17) were discharged free from hyperhidrosis for 3 months and all were still dry after 6 months.

However, disappointingly transient periods of hypohidrosis followed axillary treatment except in one case.

All patients experienced some effects of systemic absorption. Marked dryness of the mouth of 6–24 h duration was a constant feature but was rarely troublesome. Difficulty in visual accommodation was noticed by one patient and in two, treatment was ultimately withdrawn because of mild abdominal discomfort and difficulty in micturition (cases 7 and 25).

Theoretically, ionization as described should not produce bromide uptake (Scott, 1955). Serum bromides were measured on six patients directly after iontophoresis on the fifth and sixth treatment and a maximum level of 10 mg/100 ml found. Irritation and sensitization were not seen.

DISCUSSION

Histological investigation of sweat inhibition 1 week after iontophoresis of saline and other solutions by Shelley *et al.* (1948) revealed keratinous blockage of the upper epidermal part of the sweat duct of similar type to that produced by formaldehyde (Papa, 1966). Gordon & Maibach (1969) demonstrated that the anhidrosis produced by tap water iontophoresis could be reduced by skin stripping and suggested that this partially removed the sweat duct obstruction.

Papa (1966) has also shown that methylene blue is deposited in the sweat duct on iontophoresis, a technique which he used to demonstrate patency of the ducts. It seems probable that the glycopyrrolate is introduced into both epidermis and sweat gland largely by this route, but the reason for its prolonged effect is unclear. That the drug may be held in the epidermis and slowly released is suggested by the increasingly prolonged effect with increasing epidermal thickness of the treated site. A dermal site of binding for such a readily water soluble compound seems less likely, particularly since oral therapy does not have such sustained effects.

Tap water iontophoresis is much less satisfactory, as continuous dryness is only achieved by repeated treatments at short intervals and only occasionally is improvement maintained more than a few days after cessation. Grice *et al.* (1972) found similar results using tap water but poldine was noticeably more effective on palms and soles though it still required twice-weekly iontophoresis. A few patients did achieve benefit lasting up to 3 months after withdrawal. However, axillary hyperhidrosis was not adequately controlled.

The present study, using glycopyrronium bromide for palmar and plantar hyperhidrosis in adults, rarely required treatment more frequently than every 4 and 6 weeks respectively.

Despite the occurrence of mild transient systemic anticholinergic symptoms this technique appears to offer a simple reliable means of therapy. Axillary hyperhidrosis would still appear to be best dealt with by local surgery.

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Clofazimine in the treatment of discoid lupus erythematosus

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SUMMARY

Clofazimine, a rimino-phenazine dye with immunosuppressive activity, was employed in the treatment of twenty-six cases of chronic discoid lupus erythematosus. Seventeen of these cases went into remission.

Clofazimine (Lamprene) is a rimino-phenazine dye originally synthesized for the chemotherapy of tuberculosis (Barry *et al.*, 1957; Barry & Conalty, 1958, 1965). Laboratory studies have shown that clofazimine possesses antimalarial activity (Sheagren, 1968). Both clofazimine (Conalty & Jackson, 1962; Conalty 1966; Conalty, Barry & Jina, 1971) and chloroquine are sequestered within macrophages. Clofazimine stabilizes lysosomal membranes and depresses phagocytosis by the macrophage (Conalty *et al.*, 1971) which in turn can result in immunosuppression (Sabet, Newlin & Friedman, 1969). Following the institution of clofazimine therapy in patients suffering from severe erythema nodosum leprosum reactions, prednisone could be withdrawn without recurrence of hypersensitive reactions (Imkamp, 1968).

PLAN OF TRIAL

A controlled trial comparing a clofazimine with a chloroquine treated group was not undertaken because of the scarcity of patients and also because of the ocular side effects of chloroquine. Accordingly, it was decided to conduct a straightforward open trial. The present series consists of twenty-six patients in whom a course of treatment has been completed.

Any case diagnosed as discoid lupus erythematosus (LE), regardless of the stage of the disease age or sex, was admitted to the series. Diagnosis was based on clinical examination. Histological confirmation of the diagnosis was employed only where doubt might exist.

No other treatment was given to the patients beyond advice about avoiding sunlight. A sun screening cream was given to some patients. The criteria used for remission were: (i) improvement in clinical appearance; (ii) fading of the erythema; (iii) (a) scarring of already chronic lesions, or (b) return to

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normal skin structure in lesions of more recent onset. Clinical progress was recorded by colour photographs taken at intervals.

As reports in the literature suggested that improvement may be expected only after a minimum of 3 months' therapy with chloroquine, this period was decided as the minimum duration of therapy; with experience this was extended to 6 months.

RESULTS

In the twenty-six cases under review there were twenty-one females and five males. The mean age was 43·6 years and ranged from 16 to 70 years, distributed as follows:

Age (years)	No. of cases
11–20	1
21–30	1
31–40	9
41–50	8
51–60	6
61–70	1

Discoid LE was present from 3 months to 27 years with a mean of 8·6 years.

Table 1 summarizes results of therapy in our first twenty-six cases. The first six cases were given clofazimine 100 mg three times weekly for 3 months. In five of these cases the condition had been present for more than 7 years, during which they had been treated with topical corticosteroids and anti-malarials. The sixth patient noted the appearance of a discoid LE lesion 1 year previously and was treated with topical corticosteroids.

On increasing clofazimine dosage to 100 mg daily, four out of seven patients went into remission. One of these was a patient who did not improve on the lower dosage. No improvement was noted in her case with the increased dose of clofazimine.

TABLE 1. Dosage, duration and results of treatment with clofazimine in patients with discoid LE

Patient group	Dose of clofazimine	No. of patients	No. of patients improved
Group 1	100 mg × 3 weekly for 3 months	6	3
Group 2	100 mg daily for 3 months	7*	5
Group 3	200 mg daily for 3–6 months	11*	7
Group 4	(a) 200 mg daily for 1 month: 100 mg daily for 4 months	3	2
	(b) 200 mg 2 for months: 400 mg for 1 month	1	0

* One of the patients in Group 2 and one in Group 3 are 'failures' from Group 1.

With a further increase to 200 mg daily, seven of eleven patients went into remission. One of these eleven was a failure in the first group and is included in the remissions in this group.

Finally, two out of four patients in a miscellaneous group went into remission. So, in summary, seventeen of these twenty-six patients went into remission.

A feature of clofazimine therapy is that not only is it taken up by the macrophages but it is also deposited in the subcutaneous fat. The presence of a store or depot of clofazimine may be evidenced by a pink colour in the skin. Observing our patients at monthly intervals after concluding therapy, six remissions were recorded between 2 and 5 months after cessation of treatment but while the presence of clofazimine could still be noted stored in the tissues.

Treatment was initiated when the patients appeared at the department, so treatment with clofazimine was begun at all times throughout the year. Reviewing the cases, those patients treated throughout the summer months did at least as well as those treated in the darker months of the year.

The degree of pink staining of the skin was more marked with the highest dosage levels. In addition, pigmentation of the lesions was recorded in three patients in group 3. Therefore, since clinical improvement seemed to be approximately as good with 100 mg daily, this is probably the optimum dose.

DISCUSSION

As a controlled clinical trial comparing results with those obtained with chloroquine was considered inadvisable, the only standard of comparison is the trend of the disease prior to clofazimine therapy. Rowell (1968) referred to it in the following terms . . . 'the untreated skin lesions of discoid lupus erythematosus tend to be persistent'. Dubois (1966a) considered that spontaneous remission occurs in about 10% of cases.

In this present series of cases the disease had persisted for 3 months at the shortest to 27 years at the longest. Mean duration was 8·6 years. No case was in process of remission at the onset of therapy.

As some cases relapsed after stopping clofazimine, but responded to a second course, it is apparent that it is not a radical cure but simply a suppressant. This is consistent with its presumed mode of action blocking the processing of antigen in the macrophages and reflects the experience of others with chloroquine (Dubois, 1966a; Pillsbury & Jacobson, 1954).

Improvement in seventeen out of twenty-six cases (65·4%) compares favourably with other therapy, especially when many of our cases had failed on chloroquine and other treatment. Dubois (1966b) reported remission in eighteen of thirty-six cases treated with mepacrine or chloroquine. Improvement was reported in the remainder but treatment was continued for 30 months in some cases. It is unlikely that such a lengthy regime of chloroquine would be considered today.

Storage in the reticulo-endothelial cells may also account for the improvement which occurred months after stopping clofazimine therapy in six patients.

Differing rates of absorption of clofazimine may influence the results. Since it is fat soluble, a diet rich in fats would probably aid absorption.

One of the cases developed systemic lupus erythematosus 9 months after concluding a course of therapy.

The side effect of pink discoloration did not disturb the patients who regarded it as a sign of improvement in their health. It is due to deposition of clofazimine in subcutaneous fat and macrophages.

The dark brown melanin-like pigmentation in the active lesions was considered objectionable by the patients, but fortunately faded with time. The three cases in which this occurred were all receiving 200 mg clofazimine daily.

Xeroderma and keratosis pilaris were observed in two cases as previously reported by Gatti (1969) in his leprosy patients treated with clofazimine.

As clofazimine has been demonstrated to be effective in one disease with auto-immune features it may well prove to be useful in others (Conalty *et al.*, 1971).

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Intralesional triamcinolone acetonide using the Port-O-Jet and needle injections in localized dermatoses

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SUMMARY

The treatment of certain localized dermatoses with triamcinolone acetonide by needle infiltration showed a success rate of approximately 75% in a group of 1046 patients, who were followed up 6 years after a single intralesional injection.

More recently the Port-O-Jet† apparatus has been used on the same range of conditions. After a good therapeutic effect had been obtained in thirty out of thirty-nine cases in a trial series, a comparison of twenty-nine dermatoses and three onychopathic cases revealed equally good results with both methods.

The main advantages of the Port-O-Jet are its speed of action together with a pronounced decrease in pain compared to conventional needle injections.

Since 1965 the author has treated about 2000 patients with localized dermatoses by means of intralesional needle injections of triamcinolone acetonide (TA). Infiltration of steroid suspensions by needle is, however, a painful procedure, particularly in keloids, psoriatic nails or anal neurodermatitis. According to Verbov & Abell (1970), painless intralesional therapy may be achieved by means of the Port-O-Jet injector, and this method therefore seemed worth a more detailed evaluation. However, in view of the good results obtained by needle infiltration, a direct comparison of the two methods used in the same types of diseases appeared necessary to ascertain that the therapeutic effect of both methods is equal. Verbov & Abell (1970) treated mainly alopecia areata and chronic discoid lupus erythematosus. This method has also been described by Abell (1972) as being suitable for the treatment of dystrophic psoriatic nails. To make a more direct comparison possible, the author treated cases similar to those treated earlier by needle infiltration.

MATERIALS AND METHODS

Triamcinolone acetonide (Squibb) for injection was used in a concentration of 5 mg/ml. The injections, which were given only once, at the first visit, generally ranged around 0·2 ml/cm^2 and did not exceed a total of 5 ml of TA suspension. In order to assess the long-term therapeutic results, a group of patients treated over a period of 4 years was subjected to a more detailed examination. These patients were followed up about every fourth month. On each occasion the current status was compared with photographs taken previously.

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† Schuco International London Ltd.

The Port-O-Jet apparatus was first used to treat a series of thirty-nine patients in order to judge the effect of this unfamiliar method of infiltration. The results of local infiltration of TA by Port-O-Jet were then compared with conventional needle infiltration in twenty-nine cases, treating symmetrical lesions or the two halves of the same lesion. Photographs were used as an additional control (Fig. 1). As in needle injections, 0·1 ml/cm^2 was given, not exceeding a total of 5 ml of TA suspension.

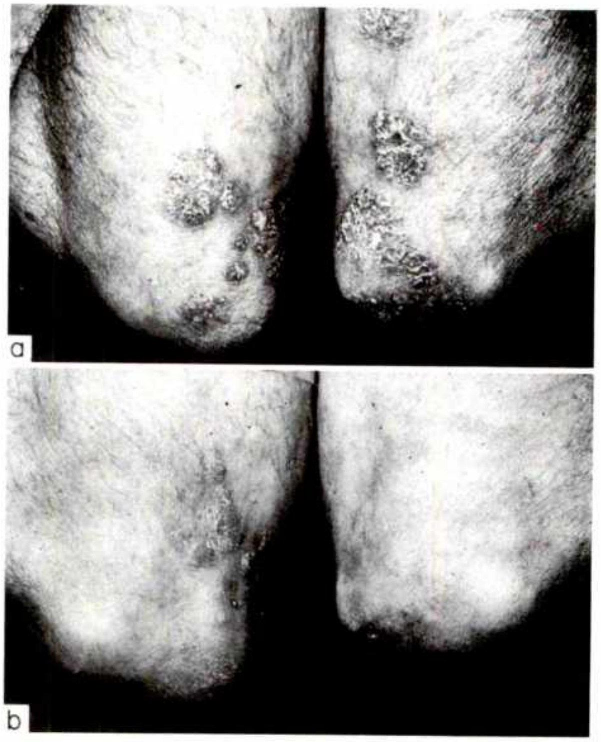

FIGURE 1. Case 15. Psoriasis. (a) Before treatment; (b) 3 months later after treatment with Port-O-Jet on left elbow (6 × 0·2 ml TA), needle infiltration (5 × 0·2 ml TA) on right elbow.

When treating psoriasis of the nails by injection, usually 0·1–0·2 ml of TA 5 mg/ml is infiltrated in each of three weals around the matrix. Since this procedure is extremely painful, the Port-O-Jet technique seemed to offer a preferable alternative; in all such cases a dose of 3 × 0·2 ml of TA was infiltrated around the base of each nail, 3–4 mm proximal to the nail plate. The infiltration was not repeated.

RESULTS

Table 1 shows the results obtained with TA needle injections. Table 2 shows that, in a selection of cases chosen with the same indications as for intralesional needle therapy, a good effect was noted in thirty out of thirty-nine patients. On comparing both methods of infiltration in the same patient,

TABLE 1. Intralesional treatment of localized dermatoses (0·2 ml triamcinolone acetonide suspension per cm²). Time of observation, 3–7 months

Treatment (15.7.1965 to 15.7.1969)	Treatment + follow-up	Healed up to June 1970	Relapse noted at follow-up, autumn 1971	Total healed
Psoriasis	610	523	56	467
Neurodermatitis				
corporis	283	254	27	227
ani	30	27	8	19
Besnier's prurigo	20	15	3	12
Keloids	39	34	6	28
Hypertrophic lichen planus	32	20	4	16
Granuloma annulare	14	12	3	9
Palmo-plantar pustulosis	20	4	3	1
Total	1048	889	110	779

the same good effect was obtained in twenty-two out of twenty-nine cases; the results obtained by both methods appeared to be the same.

A comparative study of three patients with psoriatic nail dystrophies (twenty-seven nails) showed that, on the whole, the results were the same for infiltration by Port-O-Jet as by needle. Continued treatment by Port-O-Jet infiltration alone in six additional cases resulted in approximately 80% success rate in the healing of the 109 infiltrated nails (Fig. 2). There seemed to be no difference in the healing of fingernails and toenails.

DISCUSSION

The present writer, who has had earlier experience of a relatively large and well-studied series of cases of localized dermatoses infiltrated by needle with triamcinolone acetonide, is of the opinion that infiltration therapy using the Port-O-Jet apparatus produces the same good results. The advantage of the Port-O-Jet apparatus is that the injections can be given quickly (about sixty injections per

TABLE 2. Infiltration of localized dermatoses by Port-O-Jet (0·2 ml triamcinolone acetonide per cm², 0·015 nozzle, no. 1 spacer)

	Isolated cases			Port-O-Jet compared to needle infiltration			
Diagnosis	Cases	Good effect	Poor effect	Cases	P-J better	P-J equally good	P-J poorer
Psoriasis	18	15	3	15	2	12	1
Neurodermatitis	9	7	2	6	—	5	1
Keloids	4	2	2	5	—	2	3
Granuloma annulare	2	2	—	—	—	—	—
Nail psoriasis	3	2	1	3	—	3	—
Lichen planus	3	2	1	—	—	—	—
Total	39	30	9	29	2	22	5

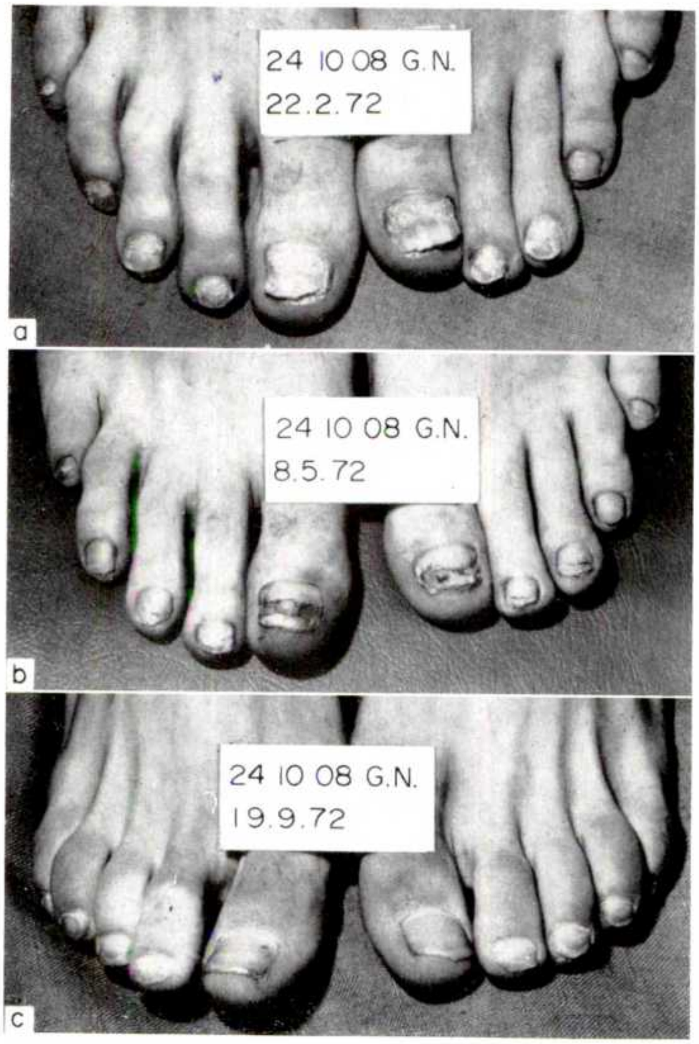

FIGURE 2. Case 6. Psoriatic nail dystrophy. (a) Before treatment; (b) and (c) 3 and 7 months respectively after infiltration of 3 × 0·2 ml TA around the base of each nail with the Port-O-Jet.

minute) and that the method causes considerably less pain at the site of infiltration. This is of great importance, for instance, in the treatment of dystrophic psoriatic nails, where needle infiltrations are hardly feasible owing to pain in the area of injection. On the other hand, psoriatic nails showing pronounced onycholysis generally showed little improvement and relapsed rapidly. Similar observations were made by Abell and Samman (1973).

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Azathioprine for pemphigus and pemphigoid—a 4 year follow-up

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SUMMARY

This is a 4 year follow-up report of our experience of azathioprine therapy in pemphigus and pemphigoid. Azathioprine produced prolonged control of the diseases in most of our twenty patients. Serious side-effects such as leukopenia were unusual, and infection was not a problem. Two of our twelve patients with pemphigoid died of cancer within 2 years of starting azathioprine. Some authors suspect that pemphigoid is related to occult or overt malignancy, and our present observation draws attention to the possibility that the risk of disseminated malignancy may be greater in patients with pemphigoid treated with immunosuppressive drugs than it would be in other non-transplant patients similarly treated.

Several authors have reported that azathioprine can be used to suppress pemphigus vulgaris (Wolff & Schreiner, 1969; Krakowski, Covo & Rosanski, 1969; Burton *et al.*, 1970; Jablonska, Chorzelski & Blaszczyk, 1970; Roenigk & Deodhar, 1973), and we also have used it successfully to treat twelve patients with pemphigoid (Greaves *et al.*, 1971). The ability of this treatment to maintain control of these diseases over several years remains to be established however, and the long-term side-effects of immunosuppressive therapy are still uncertain, particularly with regard to the risk of malignancy. Immunosuppressed patients receiving renal transplants certainly have an increased risk of developing malignancy (Hoover & Fraumeni, 1973), especially brain lymphoma (Schneck & Penn, 1971; Skinner & Schwartz, 1972), but there are grounds for believing that the transplanted organ might itself be responsible for producing the malignancy (Armstrong *et al.*, 1970; Schwartz, 1972). There is still little or no evidence to suggest that patients receiving immunosuppressive therapy for other diseases will have an increased incidence of neoplasia, and the need for more follow-up studies of patients in the non-transplant situation has recently been stressed (NIH Conference, 1972; Skinner & Schwartz, 1972). Patients with pemphigus or pemphigoid are of special interest in this respect because of the suspicion that these diseases may occasionally be related to occult or overt malignancy (Parsons & Savin, 1968; Saikia & MacConnell, 1973; Saikia, Mackie & McQueen, 1973; Saikia, 1973; Chang *et al.*, 1973). Accordingly, we here report our experience of 4 years' immunosuppressive therapy in pemphigus and in pemphigoid.

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METHODS AND RESULTS

We have used immunosuppressive treatment in the management of pemphigus and pemphigoid since April 1969. Azathioprine in a dose of 2·5 mg/kg daily has been the drug of choice since it appears to have a better therapeutic index than other immunosuppressive drugs (Corley, Lessner & Larsen, 1966; Bach, Dardenne & Fournier, 1969). In every patient Hb, WBC, ESR, blood urea and liver function tests have been monitored at intervals of 2–6 weeks.

PEMPHIGUS

In 1970 we reported a prospective trial of the use of azathioprine in four patients with pemphigus vulgaris (Burton *et al.*, 1970). One patient was unable to tolerate azathioprine, but the other three patients had an excellent response, and we were able to withdraw steroids completely. The subsequent progress of these patients (cases 1–4) is shown below, together with details of four other patients with pemphigus of various types whom we have subsequently treated with azathioprine (cases 5–8).

Case 1 is a 57-year-old man, maintained on about 40 mg prednisone daily since 1963, with bilateral ischaemic necrosis of the femoral head due to steroid therapy. Four months after starting azathioprine in April 1969 he discontinued steroids, and has remained well-controlled ever since by azathioprine, but he relapses if this is discontinued. He has had no side-effects except a normochromic anaemia (Hb 9 g/100 ml), and he remains well on azathioprine 50 mg daily.

Case 2 is a 44-year-old man maintained on about 20 mg prednisone daily since 1964, who had had severe GI bleeding and ischaemic necrosis of the femoral head due to steroid therapy. He started azathioprine in April 1969, and 8 months later he was able to discontinue steroids. He has since remained well-controlled apart from occasional mouth ulcers, but relapses if azathioprine is reduced below 75 mg daily. There have been no side-effects, but he developed acute glaucoma in October 1972.

Case 3 is an 80-year-old woman who had active umbilical pemphigus despite treatment with about 10 mg prednisone daily since 1963. Azathioprine was started in April 1969, and steroids were discontinued 6 months later. The umbilical lesions healed, and azathioprine was discontinued in April 1970. There has been no relapse.

Case 4 is a 67-year-old woman with active oral and umbilical pemphigus despite treatment with about 15 mg prednisone daily since 1966. She had adrenal atrophy, osteoporosis and collapsed vertebrae due to steroid therapy. Azathioprine, started in April 1969, was discontinued due to myalgia, vomiting and a maculo-papular rash. Cyclophosphamide was started in March 1970 but leukopenia (WBC$<3000/\text{mm}^3$) developed with doses above 25 mg daily. The disease is now controlled on prednisone 5 mg daily and cyclophosphamide 25 mg daily.

Case 5, an 87-year-old man, was maintained on about 10 mg prednisone daily since 1961. Azathioprine, started in 1969, produced a marked pancytopenia with a raised ESR (140 mm/h). Only partial haematological recovery occurred when azathioprine was stopped and he still has a raised ESR, with a moderate normochromic anaemia and leukopenia. Investigations, including sternal marrow examination, have shown no cause for this, and his pemphigus is now well-controlled by prednisone 5 mg daily.

Case 6 is an 80-year-old woman who presented in 1970 with pemphigus foliaceus and was treated with azathioprine only. This produced partial control, but was discontinued after 3 months due to severe pancytopenia, which recovered after 2 weeks. Thereafter even 50 mg azathioprine daily induced malaise and leukopenia, and she has since been maintained on prednisone.

Case 7 was a 58-year-old woman who presented in 1970 with pemphigus erythematodes and was treated with azathioprine only. This initially produced partial control but it relapsed in July 1971 while taking azathioprine 150 mg daily and again in May 1972. Each time the condition responded to prednisone, and azathioprine has now been discontinued.

Case 8 is a 46-year-old woman, now maintained on prednisone, who could not tolerate azathioprine due to nausea and vomiting.

It will be noted that cases 1–3, who responded well to azathioprine, have remained under good control for 4 years with only minor side-effects, and case 3 appears to have gone into permanent remission. Case 4, who developed a hypersensitivity reaction to azathioprine, has subsequently been controlled with cyclophosphamide and a small dose of prednisone. In case 7, azathioprine, in the dose we used (2·5 mg/kg body weight), did not suppress the disease; and cases 5, 6 and 8 were unable to tolerate azathioprine due to side-effects. In three of our eight patients with pemphigus therefore, azathioprine has produced prolonged control of the disease, in one patient it has proved inadequate to control the disease, and a further four patients have been unable to tolerate the drug. None of these eight patients, as far as we know, has developed malignancy, though the cause of the raised ESR and persistent leukopenia in case 5 remains obscure.

PEMPHIGOID

In 1971 we reported the use of azathioprine in twelve patients with pemphigoid (Greaves *et al.*, 1971). In nine of these patients corticosteroid therapy was withdrawn and the patients were maintained symptom-free on azathioprine alone, while in two the dose of prednisone was considerably reduced. Another patient who had never received corticosteroids was controlled by azathioprine alone. The subsequent progress of these patients is shown below.

Case 1 was a woman who developed pemphigoid in 1969. Treatment with prednisone produced diabetes mellitus, pneumonia and a psychosis. Azathioprine was started in April 1969, and she remained well when prednisone was stopped. In September 1970, ascites was noted and a laparotomy revealed disseminated adenocarcinoma (primary site unknown). She died of carcinomatosis in March 1971, aged 62.

Case 2 was a woman who had a mastectomy for breast cancer in 1968 and developed secondary deposits in bone soon afterwards. In May 1970 she presented with pemphigoid and, with the agreement of the radiotherapists who were managing her cancer, azathioprine was added to her oestrogen therapy, since it was felt that the cytotoxic effect of the azathioprine might outweigh any deleterious effects from immunosuppression. Azathioprine controlled the pemphigoid, but by January 1971 the bone metastases were enlarging, so azathioprine was replaced with prednisone. She continued to deteriorate steadily and died in May 1973, aged 58.

Case 3 was a man whose pemphigoid was controlled by prednisone. He started to take azathioprine in May 1969 and discontinued steroids 6 months later. The pemphigoid relapsed in 1970 after azathioprine was temporarily discontinued, but when azathioprine was again stopped in February 1971 he remained well. He died of a cerebro-vascular accident (no autopsy) in November 1971 at the age of 81.

Case 4 was a man whose pemphigoid was only partially controlled by prednisone 15 mg daily. He discontinued prednisone 4 months after starting azathioprine in May 1969, and remained well until June 1970 when he died of a myocardial infarct at the age of 88.

Case 5 is a 63-year-old woman, the control of whose pemphigoid had required large doses of prednisone since 1968, as a result of which she had ischaemic necrosis of a femoral head. Azathioprine was started in April 1969 but she was unable to discontinue prednisone until April 1970. In September 1970 azathioprine was discontinued and she relapsed 8 months later. Azathioprine and prednisone were used to achieve control, and on stopping prednisone in September 1971 she remained well. In January 1972 she developed herpes zoster of the trunk. Azathioprine was stopped and 9 months later the pemphigoid relapsed again. This was controlled by azathioprine alone and she is now well on azathioprine 150 mg daily.

Case 6 is a 53-year-old woman maintained on prednisone since 1968. Azathioprine was started in April 1969 and she remained well when steroids were discontinued. The pemphogoid relapsed in October 1969 when azathioprine was temporarily discontinued, but azathioprine again controlled the condition. Azathioprine was stopped in March 1970 and she has since remained well apart from mild transient urticated lesions.

Case 7 is a 67-year-old man who had required about 20 mg prednisone daily since 1967. Azathioprine was started in April 1969 and he remained well when steroids were discontinued 3 months later. Azathioprine was stopped in November 1969 and there has been no relapse.

Case 8 is a 73-year-old female maintained on about 5 mg daily since 1969. Azathioprine was started in April 1970 and she remained well when steroids were discontinued 3 months later. Azathioprine was discontinued in August 1970 and there was no relapse until 8 months later. This mild relapse was controlled by 5 mg prednisone daily, and azathioprine has not been restarted.

Case 9 is a 69-year-old woman who presented in September 1969 and was treated with prednisone and azathioprine. She remained well when steroids were withdrawn, but azathioprine had to be discontinued in February 1970 due to diarrhoea and vomiting. The pemphigoid relapsed 4 months later and was controlled with prednisone and cyclophosphamide. All treatment was stopped in September 1970, and she has since remained well.

Case 10 is a 69-year-old woman who had received about 20 mg prednisone daily since 1960, with consequent osteoporosis. Azathioprine was started in February 1970 but the pemphigoid relapsed when prednisone was decreased to 12·5 mg daily. She relapses also on 15 mg prednisone daily if azathioprine is discontinued. She is currently well, taking azathioprine 150 mg daily and prednisone 10 mg daily.

Cases 11 *and* 12 responded well to azathioprine and were able to discontinue steroids, but they have left the district and are lost to follow-up.

The subsequent progress of these twelve patients with pemphigoid, who all responded well to azathioprine initially, may thus be summarized as follows. Cases 1 and 2 have died of carcinoma, aged 62 and 58 respectively, after taking azathioprine for less than 2 years. Cases 3 and 4 have died, at the ages of 81 and 88, of unrelated non-malignant disease after azathioprine had controlled their pemphigoid for 30 and 13 months respectively. Cases 5, 6, 7 and 8 have either remained well-controlled by azathioprine without side-effects, or have gone into remission. Case 9 became intolerant of azathioprine after 5 months treatment, but has subsequently gone into remission after 4 months' treatment with cyclophosphamide. Case 10 had only a moderate response to azathioprine, but was able to reduce her maintenance dose of steroids by about half. Two cases have been lost to follow-up.

DISCUSSION

The present study has shown that azathioprine alone is capable of controlling pemphigus and pemphigoid for periods of at least 4 years. In some patients it is possible to reduce the dose of azathioprine progressively, and several of our patients have apparently gone into remission and have remained well for periods of up to 3 years after stopping azathioprine. The incidence of infections was low. One pemphigoid patient (case 5) developed herpes zoster but she recovered in the usual time after azathioprine was stopped.

Against this satisfactory therapeutic result must be balanced the relatively high incidence of intolerance of the drug, and the important theoretical possibility of an increased risk of developing malignancy. Of the twenty patients reported here, five (25%) had to discontinue azathioprine because of either leukopenia or nausea and vomiting, and a further two patients (both with pemphigoid) died of carcinoma. Lim, McDonald & Rook (1968) found no evidence that pemphigoid and malignancy were more than fortuitously associated in their series of 103 cases, but many of their cases were lost to follow-up, and the subject remains controversial.

According to the immunological surveillance theory (Burnet, 1970), the immune system destroys neoplasms as they arise in the body, and immunosuppressive therapy would be expected to be associated with an increased incidence of malignancy. Apart from the special case of transplant patients, however, there is at present little evidence to support the suggestion that chronic immunosuppression increases the risk of malignancy. A study of the world literature on the use of azathioprine in various immunological diseases yielded only three cases of neoplasia in over 4000 patients, compared with three cases in a smaller number of placebo-treated controls (McEwen & Petty, 1972). The correlation between therapeutic effect and immunosuppression is poor, however (NIH Conference, 1972), and it may be that azathioprine in the standard dose of 2·5 mg/kg does not produce true central immunosuppression, but suppresses antibody-mediated tissue injury by its anti-inflammatory properties. This idea is supported by the study of Denman, Denman & Greenwood (1970) who found that in patients with rheumatoid arthritis, azathioprine in a dose of 2·5 mg/kg did not suppress lymphocyte transformation or secondary antibody responses, although the drug did have profound biological effects including lymphopenia and a reduction in serum levels of immunoglobulin and of rheumatoid factor. Denman *et al.* (1970), Dodson & Bennett (1969) and Page (1962) have also shown that azathioprine and its *in vivo* metabolite, 6-mercaptopurine, suppress the activity of blood mononuclear cells, which are thought to be the precursors of the marrow-derived macrophages, which mediate chronic inflammation. It remains uncertain whether azathioprine exerts its beneficial effect in pemphigus and pemphigoid by an immunosuppressive or anti-inflammatory mechanism.

In our present study, one patient developed carcinomatous ascites 17 months after starting azathioprine, and another patient, known to have stable metastases in bone deteriorated more rapidly within a year of treatment. Thus two of our twelve patients (16%) with pemphigoid died of cancer within 3 years of starting azathioprine. In the series of Lim *et al.* (1968), only three of 103 pemphigoid patients treated with steroids alone died of cancer within 5 years. No firm conclusion can be drawn from this, but our data draw attention to the possibility that the risk of disseminated malignancy may be greater in patients with pemphigoid treated with immunosuppressive drugs than it would be in other non-transplant patients similarly treated. A similar risk might arise in premalignant conditions such as ulcerative colitis treated with immunosuppression. There are very few data on this point at present, but Korelitz, Glass & Wisch (1973) have published a report of twenty-five patients with ulcerative colitis treated with 6-mercaptopurine for up to 4 years. None of their patients developed cancer, despite the fact that they had chronic ulcerative colitis in which the risk of development of carcinoma of the rectum or colon is already high. Walder, Robertson & Jeremy (1971) have, however, found an increased incidence of skin cancers in a series of Australian patients receiving immunosuppressive therapy for renal transplants, and they felt this was related to the high prevalence of actinic keratoses in these patients before treatment. They postulated that these premalignant lesions had undergone malignant transformation more readily during the period of immunosuppression.

Our experience to date with azathioprine thus suggests that it is effective against pemphigus and pemphigoid on a long-term basis, but prolonged controlled trials are required to establish whether it is preferable to the known hazards of steroid therapy.

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Comment

RECURRENT HERPES SIMPLEX VIRUS INFECTIONS

Members of the herpes group of viruses that naturally infect man persist in the host following infection; this is as true for varicella-zoster virus, cytomegalovirus and Epstein–Barr virus as it is for herpes simplex virus. The primary infection with herpes simplex virus is most often subclinical and is followed by the emergence of antibody and latency of the virus. In a proportion of those infected the virus can be reactivated and thus give rise to recurrent lesions, the first few of which may be accompanied by a rise in antibody; thereafter, however, the appearance of lesions is unrelated to the antibody titre.

Doubt still exists as to the site of virus latency: that this is the posterior root ganglia is supported by the high incidence of herpetic eruptions over the area supplied by the second and third divisions of the fifth cranial nerve following sectioning of its posterior roots, and by the isolation of virus from the trigeminal ganglion after death (Baringer & Swoveland, 1973). Attempts to isolate the virus, during periods of quiescence, from the site of recurrences have been unsuccessful, and grafting experiments involving similar areas of skin have failed to prove that the virus is latent in this tissue. An alternative suggestion is that recurrences, especially those in the region of the nose and mouth, are really re-infections, for it has been shown that virus can be isolated from the throat and lachrymal secretions of apparently normal individuals.

Recurrent lesions, as typified by the cold sore, are generally mild and commonly occur at mucocutaneous junctions. The site of the lesion, its duration and the interval between attacks are variable, but all these factors show a degree of consistency in any one individual. A wide variety of stimuli are capable of initiating a recurrence: fever is the best known but cold, ultraviolet light, physical trauma, menstruation and emotional stress are other well authenticated precipitating factors.

Although gingivo-stomatitis is the commonest clinical presentation of a primary infection with herpes simplex virus, recurrent mouth ulcers are seldom the result of herpetic infection. Mouth ulcers may, however, be a manifestation of erythema multiforme, a hypersentitivity reaction which occasionally occurs 7–10 days after the recurrence of an herpetic lesion in an adjacent area.

Recurrent infections of the eye take the form of dendritic ulcers or stromal keratitis, but follicular conjunctivitis, which is a feature of primary ocular herpes, is uncommon. Amoeboid ulcers are most frequently seen as the result of local corticosteroid therapy for simple dendritic ulcers. They present problems in treatment and have a high incidence of serious complications. Stromal keratitis is a hypersensitivity reaction to virus antigens present in the epithelium or deeper layers of the cornea and may require corticosteroid therapy. However, this should be undertaken only with caution, under cover of antiviral chemotherapy, and when the corneal epithelium is intact (Patterson & Jones, 1967).

Disseminated lesions are unusual in normal persons with recurrent herpes, and such infections, when associated with atopic eczema, commonly lack the marked constitutional disturbance seen in eczema herpeticum resulting from a primary infection. Most cases of herpes simplex encephalitis are primary infections but, as the diagnosis has been confirmed in those with a history of cold sores or with resting antibody, reactivation of virus cannot be excluded as a possible mode of infection.

Strains of herpes simplex virus can be divided into two types on their biological and serological characteristics. Genital herpes is commonly the result of type 2 infection, whereas type 1 virus is responsible for the majority of all other lesions except those in the newborn. Recurrent genital herpes can be tiresome in either sex, but it assumes greater importance when it occurs late in pregnancy. Neonatal herpes is commonly contracted in the birth canal when the mother is suffering a primary infection. Under these conditions the foetus lacks the maternal antibody which could protect it or limit the infection resulting from recurrent herpes in the mother. On the rare occasions when disseminated lesions appear in the newborn of a mother with recurrent genital herpes it has been postulated that the absence of IgA, which is unable to cross the placental barrier, is responsible.

Although recurrent herpes is generally mild, marked extension of the lesions can undoubtedly follow the use of corticosteroids, both in the treatment of skin lesions and of those of the eye. Similarly, recurrences may become serious illnesses in those with an abnormal or depressed immune mechanism. It would appear that cell mediated immunity is of greater importance than humoral in preventing the extension of herpetic lesions (Allison, 1973). In addition to those suffering from certain naturally occurring immune deficiency diseases, e.g. Wiskott–Aldrich syndrome (but not aggammaglobulinaemia), patients with malignant diseases, especially those affecting the lymphatic system, and those receiving immunosuppressive or cytotoxic drugs are at greater risk. Recent advances in transplant surgery have increased the number of those who are highly vulnerable to extensive herpetic lesions and these infections have been responsible for, or contributed largely to, the death of a number of transplant recipients (Montgomerie *et al.*, 1969). In these patients, recurrences are not necessarily more frequent, but a simple cold sore is liable to extend to the oral cavity and may progress to give rise to oesophagitis and even pneumonitis. Early recognition of the condition is important, for viral chemotherapy can be lifesaving.

Two compounds, idoxuridine and cytosine arabinoside, are of proved value in the treatment of herpes simplex infections. Because primary infections are generally the more severe, the most dramatic effects of viral chemotherapy are seen in these illnesses. However, these compounds are equally as valuable in the treatment of the extensive lesions of recurrent infections in immunosuppressed patients, and it is claimed that they reduce the frequency, as well as shorten the duration, of recurrences in the milder forms of the disease.

Idoxuridine (5-iodo-2′-deoxyuridine, IDU) is a synthetic, halogenated analogue of thymidine which is incorporated into the viral DNA to form a defective molecule. It is relatively insoluble in water but can be dissolved in dimethyl sulphoxide (DMSO) and in this vehicle has been used in the treatment of skin lesions (MacCallum & Juel-Jensen, 1966). Applications are limited to 3–4 days, as more prolonged use leads to maceration of the skin and predisposes to secondary bacterial infection. Treatment needs to be commenced early, when tingling and itching are present, and before the eruption is apparent. Idoxuridine may be effective in recurrent genital herpes and if early and intense treatment of the herpetic lesion is undertaken in those susceptible to erythema multiforme, the hypersensitivity reaction can be aborted or modified.

Simple dendritic ulcers and those with disciform keratitis respond equally well to idoxuridine or cauterization. Idoxuridine is the treatment of choice in amoeboid ulcers, in dendritic ulcers in children who would need an anaesthetic for accurate cauterization, and in patients whose ulcers would necessitate a large area of cauterization.

Cytosine arabinoside (1-*β*-D-arabinofuranosylcytosine, cytarabine) differs from the natural nucleoside, cytidine, in possessing arabinose instead of ribose as the sugar moiety. It is effective, *in vitro*, against a number of DNA viruses, including herpes simplex virus, and is relatively non-toxic to those with a normal bone marrow, although marrow depression with leukopoenia, anaemia and thrombocytopoenia can follow its administration. Because it is rapidly deaminated, it has been suggested that the daily dose should be given rapidly in one injection. This antiviral compound has been used successfully in the treatment of extensive recurrent herpes and, because these infections are potentially so serious in those receiving organ transplants, it has been recommended that cytosine arabinoside should be given until virus can no longer be recovered or until bone marrow depression occurs.

Adenosine arabinoside (9-β-D-arabinofuranosyladanine) has a greater therapeutic index than cytosine arabinoside because the first metabolic product still possesses antiviral activity. As a smaller dosage may be effective, adenosine arabinoside could prove to be the drug of choice in treating those patients whose bone marrow is already depressed. A recent report of the treatment of ten immunosuppressed patients (Ch'ien *et al.*, 1974) is encouraging, although it was observed that type 2 infections responded more slowly and less completely than type 1 infections.

There is little evidence that interferon plays anything but a minor role in recovery from recurrent herpes. Inactivated vaccines are of doubtful benefit and can lead to hypersensitivity reactions, whereas live vaccines are dangerous and should not be given. However, photodynamic inactivation of herpes simplex virus by the application of neutral red to the base of the lesions, followed by exposure to flourescent or incandescent light, has been shown to produce symptomatic improvement, reduce healing time and increase the interval between attacks in recurrent lesions of both type 1 and 2 infections (Felber *et al.*, 1973).

P.G.Higgins

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British Journal of Dermatology (1974) **91**, 115.

Correspondence

ALLOPURINOL AND DRUG INDUCED ALOPECIA

SIR, In their review of 'Drug induced alopecia', Levantine & Almeyda (1973) refer to a previous report by Auerbach & Orentrich (1968) and write '. . . its (allopurinol) action could be regarded as cytotoxic'.

The evidence does not support such a statement.

Allopurinol inhibits the action of xanthine oxidase (Elion, 1966), the enzyme which facilitates the final steps of purine catabolism:

$$\text{Hypoxanthine} \xrightarrow{\text{Xanthine oxidase}} \text{Xanthine} \xrightarrow{\text{Xanthine oxidase}} \text{Uric acid}$$

I know of no evidence that xanthine oxidase is involved in purine anabolism. In fact, the disease process xanthinuria, which is characterized by a deficiency of xanthine oxidase activity, supports the concept that xanthine oxidase activity is not essential for human life (Sperling, 1970).

Prior to its introduction as a drug for its present use, allopurinol was considered as a possible cytotoxic agent. Deliberate and intensive study showed it to have no such activity (Rundles *et al.*, 1963; Hitchings, 1966).

Eight years of world-wide experience with allopurinol in the treatment of gouty arthritis has produced no evidence of cytotoxic activity.

Hence I suggest allopurinol should not be regarded as cytotoxic.

The Wellcome Foundation Ltd,
London, N W.1

G.E.LOVATT

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PREVALENCE OF STREPTOCOCCI AND STAPHYLOCOCCI IN LESIONS OF IMPETIGO

SIR, In 1972, one of us (B.L.C.) reported on the prevalence of streptococci and staphylococci in lesions of impetigo in the U.K. There was a low prevalence of streptococci (21%) which, although in accord with other surveys in the U.K., was at variance with reports from other countries, especially the U.S.A., in which streptococcal prevalence rates of more than 70% have been recorded (Dillon, Reeves & Maxted, 1968; Ortiz *et al.*, 1970; Dajani, Ferrieri & Wannamaker, 1972).

In 1973, Taplin & Lansdell reported higher recoveries of beta haemolytic streptococci from swabs dried in silica gel as compared with the more conventional use of Stuart's Transport Medium. It seemed possible, therefore, that some streptococci were missed in the study reported by Connor (1972).

TABLE I. Flora of impetigo lesions

Organism	Swabs processed in London	Swabs processed in Miami
Staph. aureus only	33	28
Beta streptococci only	2	7
Staph. and strep.	12	6
Neither	3	9
Total	50	50

Accordingly, we have re-examined the bacteriology of impetigo in the U.K., taking samples from patients in London and in the Edinburgh area. All examinations were carried out in duplicate. One cotton swab was sent in Stuart's Transport Medium to a laboratory in London and processed as reported previously (Connor, 1972) and one alginate swab was posted to Miami in silica gel. The results are shown in Table 1. There is no evidence from these samples that streptococci in skin lesions are missed by using Stuart's medium, during the short time (about 3 days) required, in the U.K., to transport the swabs to the receiving laboratory, and it is clear that in the superficial impetigo seen in Britain, *Staph. aureus* is the pathogen most commonly recovered.

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Book Reviews

Contact Dermatitis. Alexander A.Fisher (1973) 2nd edition. Philadelphia: Lea and Febiger (U.K. distributors: Kimpton). Pp. xiv + 448, illustrated, 14 colour plates. Price £8.75.

The first edition of Dr Fisher's well-known book on contact dermatitis was published in 1967 and ran to 324 pages. The expansion of the second edition to 448 pages reflects not only the growing importance of the subject and the voluminous literature it engenders, but, more significantly, the more international approach which the author has rightly chosen to adopt. As he acknowledges, he owes this broader view to the North American Contact Dermatitis Research Group, which maintains a close and friendly association with Charles Calnan's International Contact Dermatitis Research Group.

The book is essentially a practical manual for daily consultation by the dermatologist in practice. It provides, in a form eminently suitable for quick reference, details of most of the substances proved to cause dermatitis, their composition where this is known, and their occurrence in the domestic, occupational or recreational environment. There are particularly useful tables listing the composition of many proprietary pharmaceutical or cosmetic preparations. However, study of these tables emphasizes impressively the striking geographical variations in the contact dermatitis problem. That this book is international in outlook has already been stated, yet it would have been entirely impracticable for Dr Fisher to have provided similar tables of the often very different composition of comparable proprietary preparations available in other countries, since no two are the same, and many differ profoundly. Any dermatologist who has not already compiled such information should show these tables to his hospital pharmacist and ask his help in obtaining particulars of the composition and popularity in his own country of the growing numbers of proprietary products, ranging from after-shave lotions to vaginal cosmetics, to which his own patients are at risk.

The reviewer was interested in some of the recommended treatments. For persistent chronic lichenified dermatitis most dermatologists in Britain would prefer a tar paste or even β radiation, to the solution of coal tar or Arning's tincture here suggested. Similarly, cleaner but equally effective preparations would be preferred to Castellani's carbolfuchsin paint in macerated intertriginous dermatitis.

In several chapters of this excellent book Dr Fisher has benefited from the advice of authorities with exceptional experience in some specialized field, for example Dr Harber on photodermatitis and Dr Orris on aquatic contact dermatitis. He would have done well to seek the advice of a botanist on his chapter on Dermatitis due to Plants and Spices, in which there are some confusing and inaccurate statements. A list on page 244 begins with a genus, followed by a species, and then continues with the names of several plant families, which in fact include both the genus and the species already mentioned. Although the Latin names of plants are usually given, in many instances these are omitted; this is regrettable since the same English word, for example, daisy, is often popularly applied to several different genera, some of which are sensitizers, but most of which are innocuous. It is ironic that as dermatologists we are prepared to accept terms such as hyperkeratosis follicularis et parafollicularis in cutem penetrans (Kyrle), yet reluctant to accept that it matters whether we refer to the 'daisy' (*Bellis perennis*) which is not a sensitizer, or to the 'daisy' of the florist (*Chrysanthemum maximum*) which is. The statement on page 252 that 'there are many varieties of primrose plants, any of which may cause dermatitis', is untrue. There are very few offenders in the large Primulaceae family, and even in the genus *Primula*, most species, including the common English primrose, have not been reliably proved to cause dermatitis. Many of the plant families listed on pages 248–9 as causing phytophotodermatitis do not do so, and this clinical syndrome is not clearly differentiated from photodermatitis following ingestion of a plant, as occurs with certain Chenopodiaceae species.

However, these criticisms are relatively trivial. This is a book which can be strongly recommended. It is a great deal more than a comprehensive and conscientious survey of a massive international literature. Every chapter is illuminated by important practical information which is the fruit of the author's vast personal experience.

ARTHUR ROOK

Current Problems in Dermatology. Volume 5. J.W.H.MALI (Ed.) (1973) Basel: Karger. Pp. viii+182, 37 figures, 26 tables. Price £12.90.

This is the third volume of Current Problems in Dermatology to be edited by Professor Mali at intervals of little over a year, since he took over responsibility for the series in 1970. It might be suggested that the literature of dermatology is so well served by review articles at all levels of scientific profundity that yet another collection is an unnecessary luxury. But this is not so, for the rapidly expanding frontiers of dermatological research encompass ever larger areas of basic science, so that each part of dermatology is at risk of becoming a subspecialty. If the timely subject and the knowledgeable and lucid reviewer are happily married a detailed and critical review can be of immense value to workers in other corners of the same field, as well as to the clinician who wishes to keep abreast of advances which have immediate or potential relevance, even at the purely practical level.

In this new volume Mali follows the formula which proved so successful in his previous volumes—he chooses the right subjects and the right authors.

J.E.Wahlberg's review of percutaneous absorption is notable for its exceptionally critical survey of definitions and criteria used by many workers, and of potentially important variables which have often not been taken into account. K.Adachi's account of the metabolism and control mechanisms of human hair follicles brings together much widely scattered work and offers a convincing hypothesis as a basis for further research. It is to be hoped that his chapter will be studied by those gynaecological endocrinologists who are investigating hirsutism and whose new findings fascinatingly complement those of Adachi.

The third chapter on physical urticaria is by Professor Illig of Giessen. The review is again comprehensive and up to date and although much of his material has, as he says, already been published, it is time that it was made freely available to those who do not read German.

The subjects of the remaining two chapters are equally well chosen, for both concern clinical entities, the variable manifestations of which may bring those afflicted with them under the care of any one of four or more specialists. Consequently the literature on each syndrome is so dispersed that few clinicians are familiar with more than their own fraction of it, and are unable to take a detached overall view. Professor Hornstein of Erlangen provides an excellent critical assessment of the Melkersson–Rosenthal syndrome, and emphasizes that it is less rare than rarely diagnosed; patients may find their way in the first instance either to a general physician, a neurologist, a psychiatrist, a stomatologist, an ear, nose and throat surgeon or a dermatologist, and no single clinic is therefore in a position to estimate the prevalence of the syndrome. Dr J.T.Sharp writes equally well on Reiter's syndrome. He writes as a rheumatologist, but these cases may also fall within the boundaries of dermatology, venereology, ophthalmology or internal medicine.

This book is strongly recommended. It is well produced, as is to be expected from Messrs Karger. The reviewer's only criticism is that the English style of some writers whose native language is not English is sometimes clumsy and confused. It could perhaps have been polished, not for any pedantic reason, but to provide greater clarity and a more relaxingly readable text.

ARTHUR ROOK

Holzarten mit Gesundheitsschädigenden Inhaltsstoffen. BJÖRN M.HAUSEN (1973) Stuttgart: DRW-Verlags-GmbH. Pp. 148. Price £2.60.

When this book was reviewed in our December issue (*British Journal of Dermatology*, 1973, **89**, 653) we were not aware that the comprehensive bibliography has been published separately. It may be ordered directly from the author of the book, Dr Hausen, Department of Dermatology, University of Hamburg, for the price of DM 5.00.

Paediatric Allergy and Clinical Immunology (as applied to Atopic Disease). C.COLLINS-WILLIAMS (1973) 4th edition. London: Churchill-Livingstone. Pp. 161. Price £4.00.

The theory and practice of 'allergy' is a closed book to many dermatologists, yet some of their patients are likely to be concurrently in the care of those practising this branch of medicine. Dr Cecil Collins-

Williams is Head of the Allergy Division of the Hospital for Sick Children at Toronto. His considerable experience in allergic disease has been distilled in this 'quick reference volume for the student or practising physician', intended to help him in his understanding, investigation and treatment of atopic disease. It has been deservedly popular, perhaps because of its synoptic presentation and didactic approach. The text is in note form, clearly set out and illustrated with diagnostic record forms and lists of causes (e.g. of urticaria in children) and of investigations required. The approach is eminently practical—the history is given pre-eminence, skin tests (especially in eczema) are treated with reserve and tonsils are not there for the taking.

The section on the diagnosis and general management of atopic dermatitis follows acceptable lines, though great emphasis is attached to ingested allergens. Dermatologists in this country may well feel that the management of the abnormal physiological responses of the atopic should be given as much space. They may also question the use of Neosporin, 'sulpha' drugs or penicillin for infected eczema, especially as the author later devotes a complete section to the dangers of penicillin.

Some 30 pages of appendix are devoted to elimination diets (including a miniature Mrs Beeton for the harrassed mother trying to turn soya and tapioca into interesting dishes). The paucity of dermatological references indicates how far allergy has become separated from dermatology in North America. Nevertheless, for the practising physician constantly faced with problems involving the atopic subject, there is much of value in this book, which should certainly find a place in every dermatologist's reference library. It is very reasonably priced by today's standards.

D.S.WILKINSON

Fortschritte der praktischen Dermatologie und Venerologie. Volume VII. O.BRAUN-FALCO and D.PETZOLDT (Eds.) (1973) Berlin: Springer. Pp. 334, 66 figures.

This volume presents the proceedings of the seventh of the annual refresher courses for dermatologists, organized by Professor Braun-Falco and his colleagues of the Department of Dermatology of the University of Munich. The main themes of the July 1973 course were skin cancer and precancer, topical therapy, phlebology, andrology, skin reactions of multiple aetiology, therapy and prophylaxis, and radiotherapy. Four or five papers were devoted to various different aspects of each main theme. The papers are short and necessarily didactic, but they effectively cover recent advances and provide up-to-date lists of references. The book, like the course on which it was based, is intended for the clinical dermatologist, and its emphasis is essentially practical. Editors and publishers are to be congratulated on securing such effective collaboration from the numerous contributors that it was possible for the book to be published within 6 months of the course; it is indeed the unusually short publication delay which gives these papers their interest and value. The articles on therapy are particularly timely and useful. It would probably be in the publishers' interest to consider the simultaneous publication of an English translation of any future courses of this quality, if this could be achieved without the delays that bedevil so many such ventures.

ARTHUR ROOK

Radiodermites des Mains. F.LAGROT (1974) Paris: Doin. Pp. 231, 82 figures.

The author, a plastic surgeon, records in this monograph his experience of over 100 cases of radiodermatitis of the hands over a period of 25 years.

By far the greatest number of these patients were doctors. Radiologists, being better aware of the dangers, form a minority and most of the patients were surgeons who had been exposed, particularly in operating under the X-ray screen for the removal of metallic foreign bodies. Physicians, especially chest physicians, form the next large group, while technicians and nurses were fewer. Only eleven cases were in patients who had received radiotherapy to the hands.

British Journal of Dermatology (1974) **91**, 121.

News and Notices

Retirement of Dr Arthur Rook as Editor of the British Journal of Dermatology

After nearly seven, very active years Dr Rook's term of office as editor has just finished. Fortunately, however, he has by no means relinquished his interest in the affairs of the Journal, or of the British Association of Dermatologists of which he has now become President.

The past seven years have seen many notable changes in the style of the Journal, also in the quantity, quality and diversity of material published.

In recognition of his outstanding services the British Association of Dermatologists have commissioned a portrait of Dr Rook and presented it to him.

British Photobiology Society

The Society is to hold a one-day meeting in London at the Edward Lewis Theatre in the Middlesex Hospital Medical School, London, W.1 on Wednesday, 23 October 1974. The meeting entitled 'Recent Advances in Ultraviolet and Visible Radiation Effects on the Skin' will include papers on UVR induced DNA repair and on clinical photobiology. Interested persons should write for a programme to Dr C.A. Ramsay, Institute of Dermatology, Homerton Grove, London E.9.

Investigative Group of the British Association of Dermatologists

The next meeting of the Group will be held at the Middlesex Hospital Medical School on Saturday, 25 January 1975. The meeting will be preceded by a dinner on the evening of Friday, 24 January. Members wishing to present papers should send standardized abstracts (details of which will be precirculated) to Dr M.W.Greaves, Department of Dermatology, Royal Victoria Infirmary, Newcastle on Tyne, not later than Monday, 14 October 1974.

Ibero Latin American Congress of Dermatology

The 8th Ibero Latin American Congress of Dermatology will be held in San Salvador, El Salvador, Central America, from 28 November to 3 December 1975.

Those interested should write to Professor Enrique Flores Diaz, General Secretary of the Congress, P.O. Box 1443, San Salvador, El Salvador, Central America.

Institute of Dermatology

(University of London)

Applications are invited from those wishing to be considered for inclusion in the Diploma in Dermatology Course commencing 1 October 1975. Applications should be submitted before 31 December 1974, and applicants will be notified in March 1975 as to whether or not their application has been successful. Application forms may be obtained from the Dean, Institute of Dermatology, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ.

FELLOWSHIPS AND AWARDS

Royal College of Physicians

The following Dermatologists have recently been elected to the Fellowship of the Royal College of Physicians, London:

J.S.Comaish, P.B.Fox, M.Greaves, G.Holti, A.Maguive, D.M.Mitchell, D.D.Munro, E.J.T. Prettejohn, T.Ryan and R.Summerly.

Dowling Travelling Fellowship

The Dowling Travelling Fellowship has been awarded to Dr E.Abell of St John's Hospital for Diseases of the Skin, London.

1973 Psoriasis Research Award

The Department of Dermatology, Baylor College of Medicine, announces that this year's Mr and Mrs J.N.Taub International Memorial Award for Psoriasis Research will go to John J.Voorhess, M.D. and Elizabeth A.Duell, Ph.D. of the Department of Dermatology, University of Michigan, for their research relating to control of epidermal cell proliferation. These individuals were selected for the award because publications resulting from their research are highly regarded throughout the scientific community and the findings have implications beyond the field of dermatology, as well as to psoriasis.

APPOINTMENTS

Derby Royal Infirmary

Dr A.Navaratnam has been appointed Consultant Dermatologist to the Derby Royal Infirmary. He qualified M.B., B.S. at the University of Ceylon in 1956, and obtained his M.R.C.P. (Edinburgh) in 1969, with dermatology as a special subject. In the same year he became M.R.C.P. (U.K.). Until 1966 he worked in various positions in Ceylon and then came to England where he did general medicine at Bury St Edmunds. In 1970 he became Registrar in Dermatology at St Mary's Hospital, Paddington. From 1971 he has been Senior Registrar in the University Hospital of Wales, Cardiff. He has written a number of case histories and discussions for the British Journal of Dermatology, he has been interested in the histology of lichen planus, and skin markers in patients with internal neoplasms.

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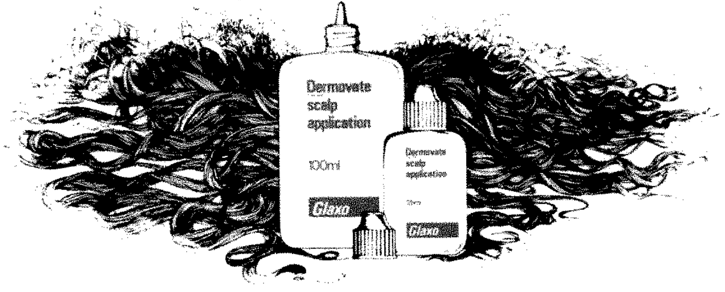

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VOL. 25 NO. 6 JUNE 1974

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R. A. COWEN. Relative merits of 'in use' and laboratory methods for the evaluation of antimicrobial products.

J. W. K. BURRELL. The behaviour of perfumery ingredients in products.

K. V. CURRY. Evaluation of skin bleach creams.

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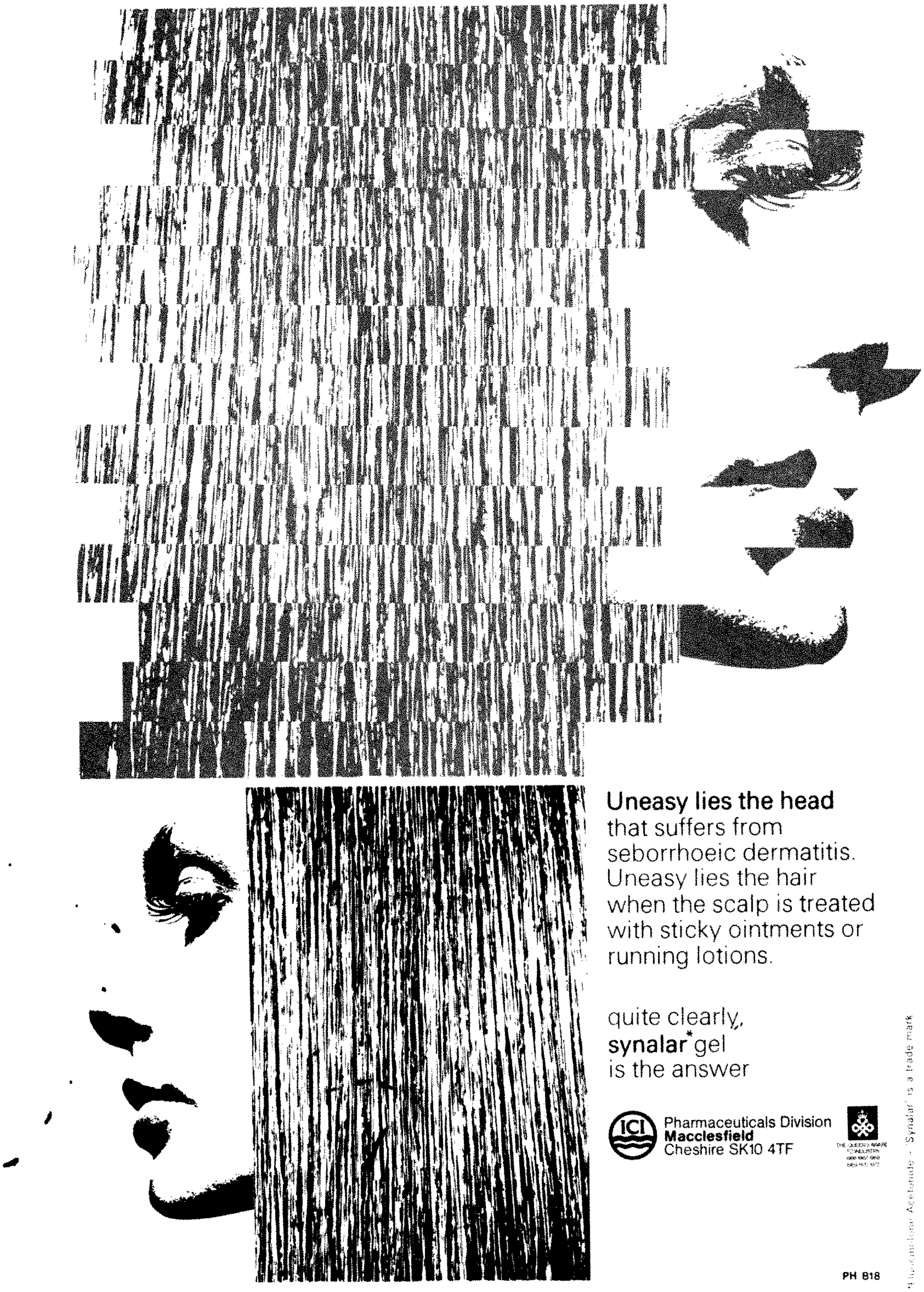

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Index Vol. 147, No. 5, 1973

Anton-Lamprecht, I. und *Schnyder, U.W.* (Heidelberg): Epidermolysis bullosa dystrophica dominans – ein Defekt der anchoring fibrils?
(Epidermolysis bullosa dystrophica dominans – a defect of the anchoring fibrils?) . . . .

Dupré, A.; Bolinelli, R.; Christol, B., and *Bonafé, J.L.* (Toulouse): La variété médiodorsale de l'infiltration lymphocytaire de la peau (Jessner et Kanof). Présentation de deux observations dont une associée à une maladie de Hodgkin
(Elective mediodorsal localization in lymphocytic infiltration of skin [Jessner and Kanof]. Report of two cases, one associated with morbus Hodgkin) . . . . . .

Tring, F.C. (Sheffield): Skin Colour Changes in Psoriasis. Effect of UV Light Irradiation . .

Levo, Y.; Pick, A.I.; Nir, M.A.; Weiss, C., and *Feuerman, E.J.* (Petah Tikva/Tel Aviv): Correlation between Immunological Parameters and Cutaneous Manifestations of Systemic Lupus erythematosus . . . .

Konstantinov, D. and *Stanoeva, L.* (Skopje): Persistent Scabious Nodules . . . .

Notowicz, A. and *Menke, H.E.* (Rotterdam): Atypical Primary Syphilitic Lesions on the Penis . . . .

Smeenk, G. (Deventer): Contact Allergy to Bufexamac . . . .

Young, E. (Utrecht): Allantoin in Treatment of Psoriasis . . . .

Singh, G. (Varanasi): Heat, Humidity and Pyodermas . . . .

The Interesting Case / Der interessante Fall / Un cas intéressant

Krakowsky, A.; Brenner, S.; Covo, J.; Loewenthal, M., and *Baratz, M.* (Tel Aviv): Acquired Ichthyosis in Kaposi's Sarcoma . . . .

Letter to the Editor . . . .

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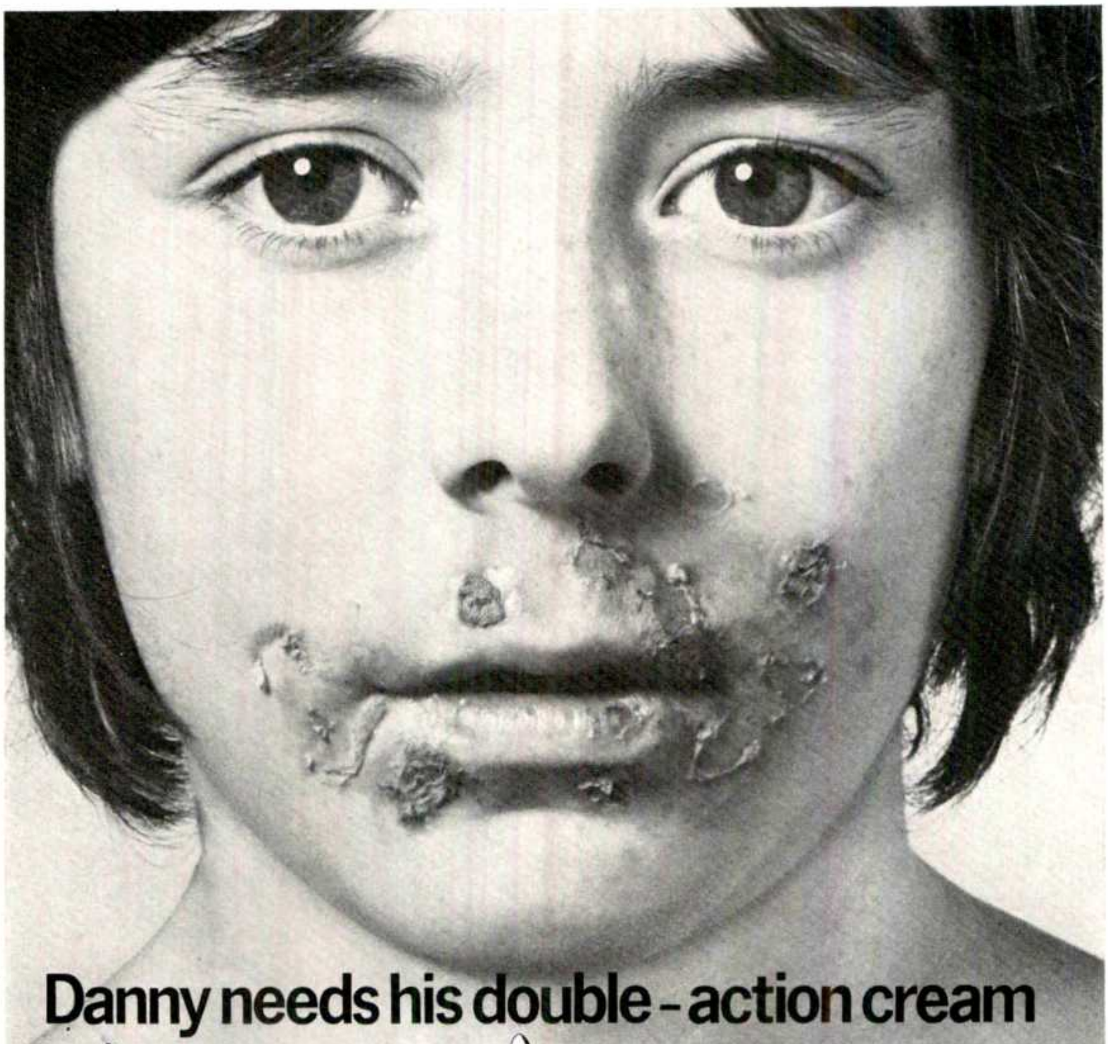

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Edited by

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F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

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British Journal of Dermatology, Volume 91, Number 1, July 1974

CONTENTS

PAGE

Clinical and Laboratory Investigations

Leukocyte Migration Inhibition in Progressive Systemic Sclerosis 1
P. HUGHES, SHIRLEY HOLT AND N. R. ROWELL

A 7-year Follow-up Study of Ninety Patients with Psoriasis 7
GILLIAN A. DURHAM AND JOHN K. MORGAN

Contact Dermatitis from Plants of the Caper Family, Capparidaceae. Effects on the Skin of Some Plants which Yield *Iso*thiocyanates 13
J. C. MITCHELL

Induction of Delayed Hypersensitivity to Nitrogen Mustard in the Guinea-pig 21
HENRY C. MAGUIRE, JR

Percutaneous Absorption and the Surface Area of Occluded Skin. A Scanning Electron Microscopic Study 27
DAVID R. HARRIS, CHRISTOPHER M. PAPA AND RAYMOND STANTON

The Influence of Enzyme Detergent on Skin Reactions to Beryllium 33
F. RAY BETTLEY AND ALAN O'SHEA

Keratinization in Dispersed Cell Cultures of Adult Guinea-pig Ear Skin 39
HILARY CONSTABLE, J. R. COOPER, C. N. D. CRUICKSHANK AND PATRICIA R. MANN

The Activity of Various Topical Griseofulvin Preparations and the Appearance of Oral Griseofulvin in the Stratum Corneum 49
ARTHUR G. KNIGHT

The Investigation of Superficial Fungal Infections by Skin Surface Biopsy 57
D. A. WHITING AND E. A. BISSET

Onychomycosis in Elderly Chiropody Patients 67
MARY P. ENGLISH AND R. ATKINSON

Glucose Tolerance in Lichen Planus 73
SHEILA M. POWELL, JULIA P. ELLIS, T. J. RYAN AND H. R. VICKERS

Differential Diagnosis of Ischaemic Ulceration of the Leg Using Ultrasound 77
J. P. WOODCOCK, SUZANNE ALEXANDER AND M. DURKIN

Lichen Sclerosus et Atrophicus in the Bantu 81
M. DOGLIOTTI, C. B. BENTLEY-PHILLIPS AND A. SCHMAMAN

Pharmacology and Treatment

The Treatment of Idiopathic Hyperhidrosis by Glycopyrronium Bromide and Tap Water Iontopheresis 87
E. ABELL AND K. MORGAN

Clofazimine in the Treatment of Discoid Lupus Erythematosus 93
J. P. MACKAY AND J. BARNES

Intralesional Triamcinolone Acetonide Using the Port-O-Jet and Needle Injections in Localized Dermatoses 97
J. J. BLEEKER

Azathioprine for Pemphigus and Pemphigoid—a 4-Year Follow-up 103
J. L. BURTON AND M. W. GREAVES

Comment ...... 11[illegible]

Correspondence

Allopurinol and Drug Induced Alopecia 115
G. E. LOVATT

Prevalence of Streptococci and Staphylococci in Lesions of Impetigo ...... [illegible]
W. C. NOBLE, D. PRESBURY, B. L. CONNOR, J. A. SAVIN, D. TAPLIN AND LYLE LANSDELL

Book Reviews ...... [illegible]

News and Notices ...... [illegible]

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Volume 91, Number 3, September 1974

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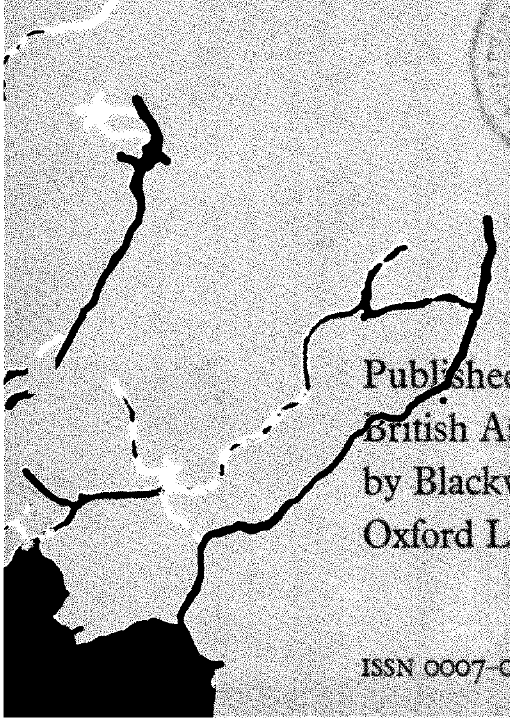

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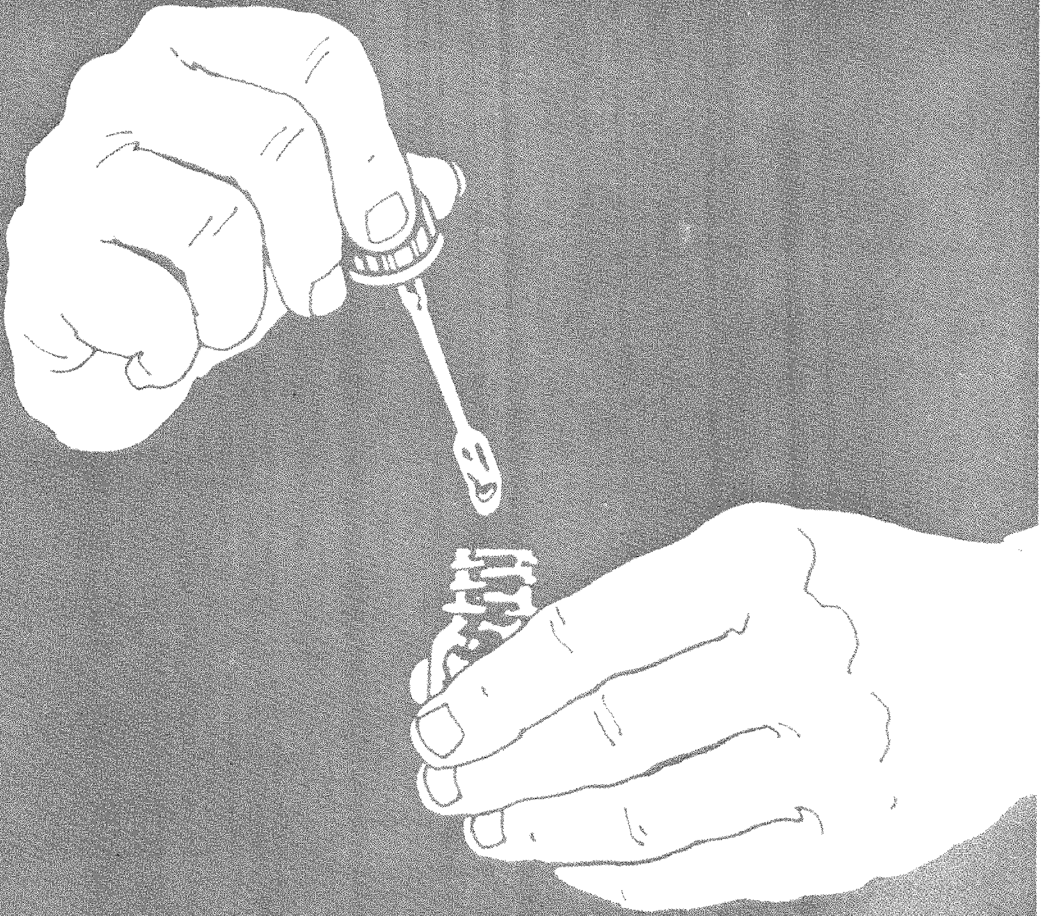

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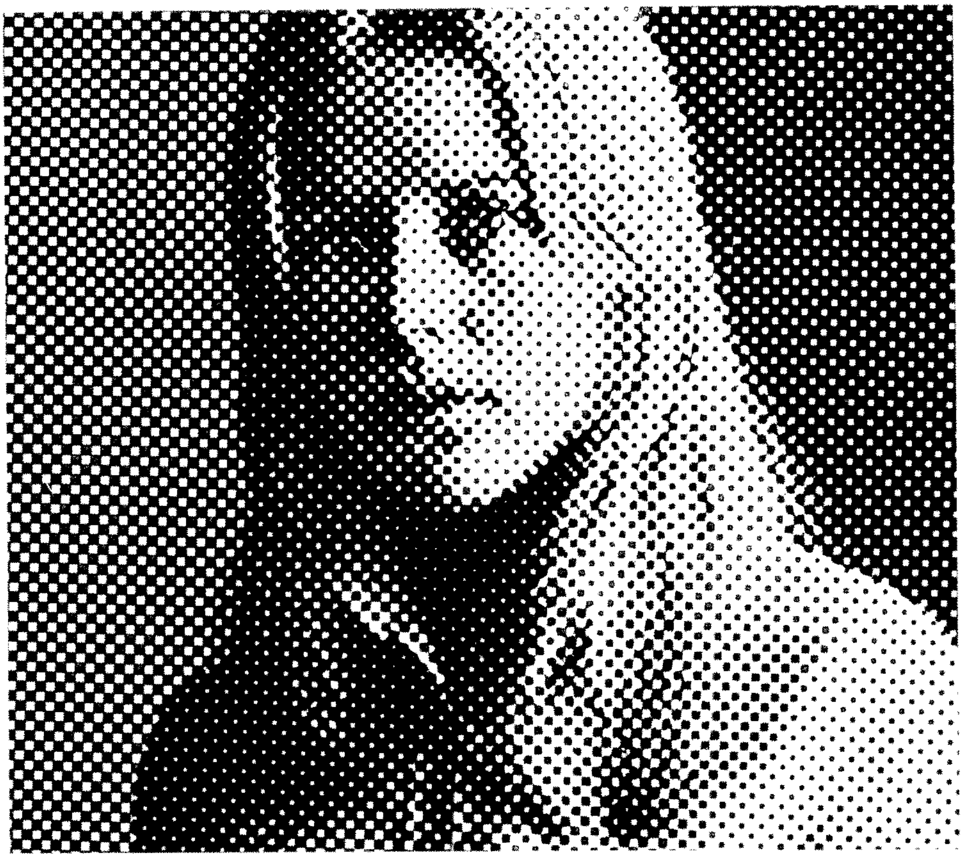

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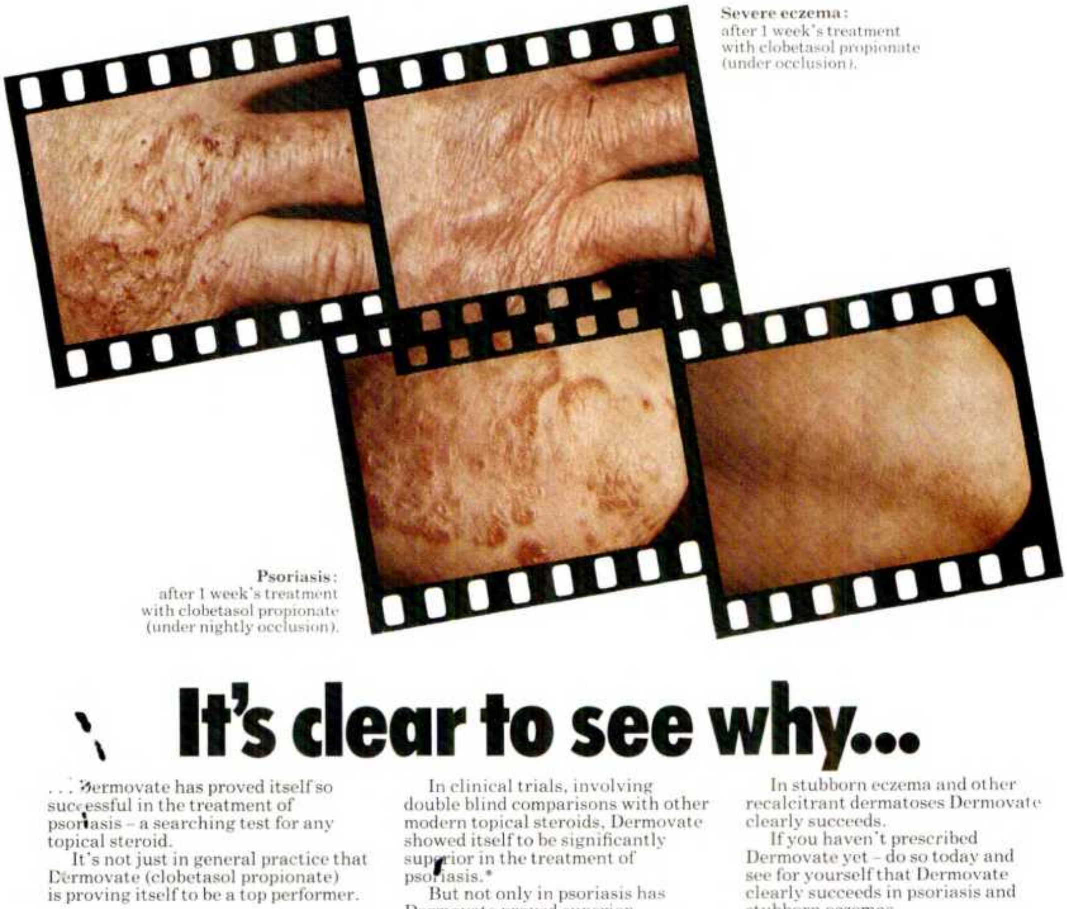

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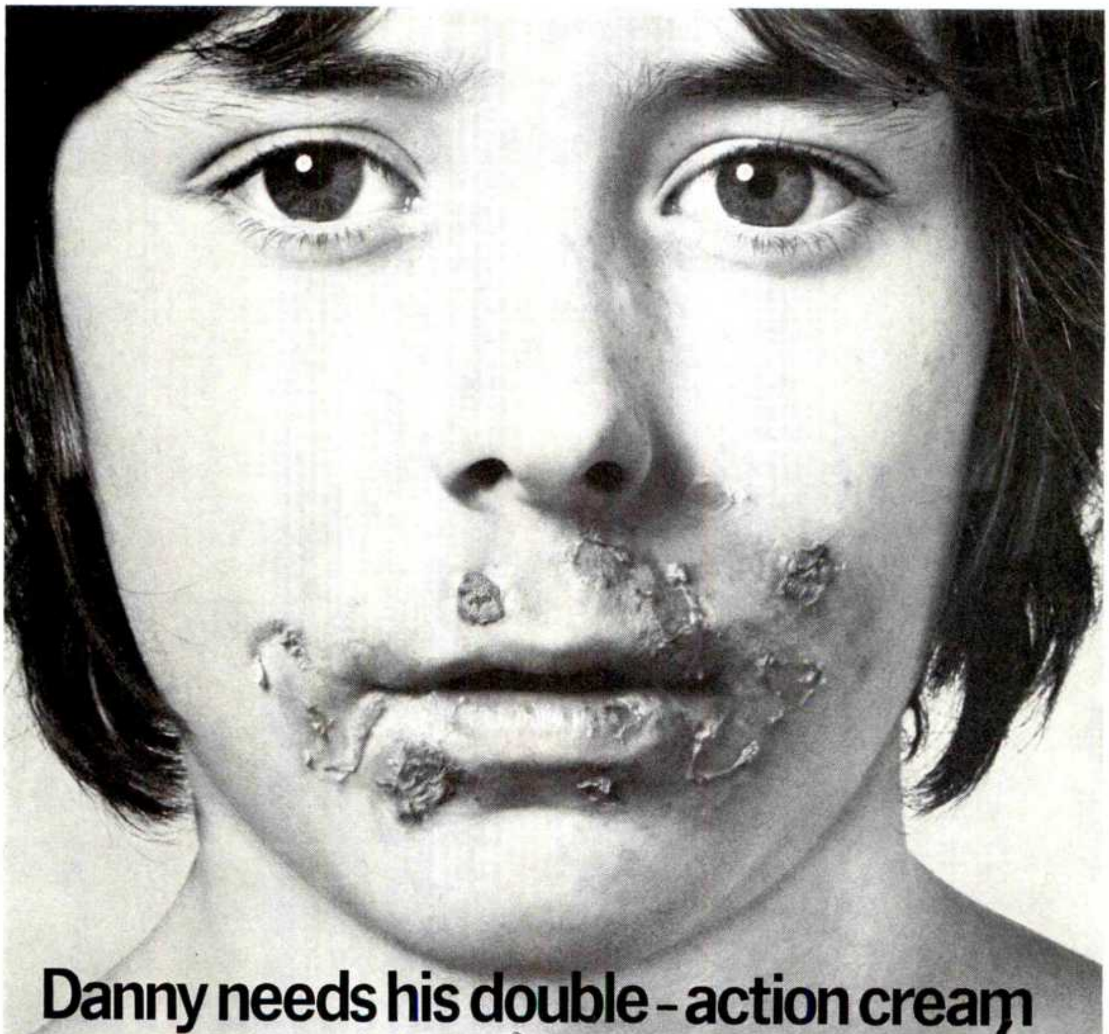

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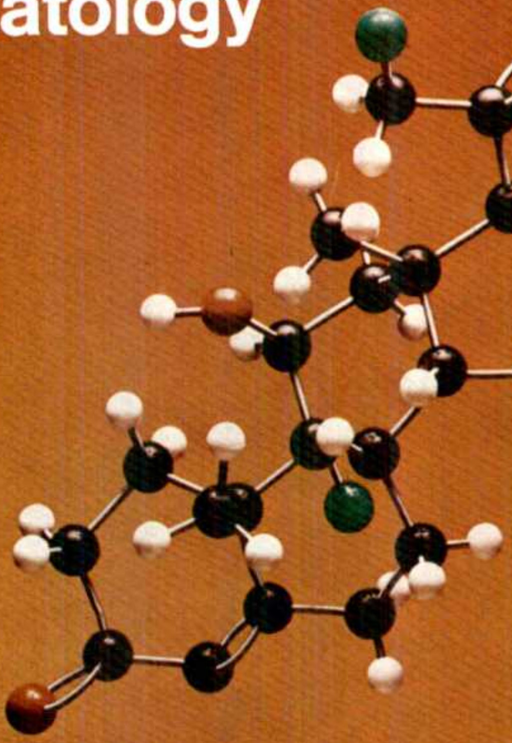

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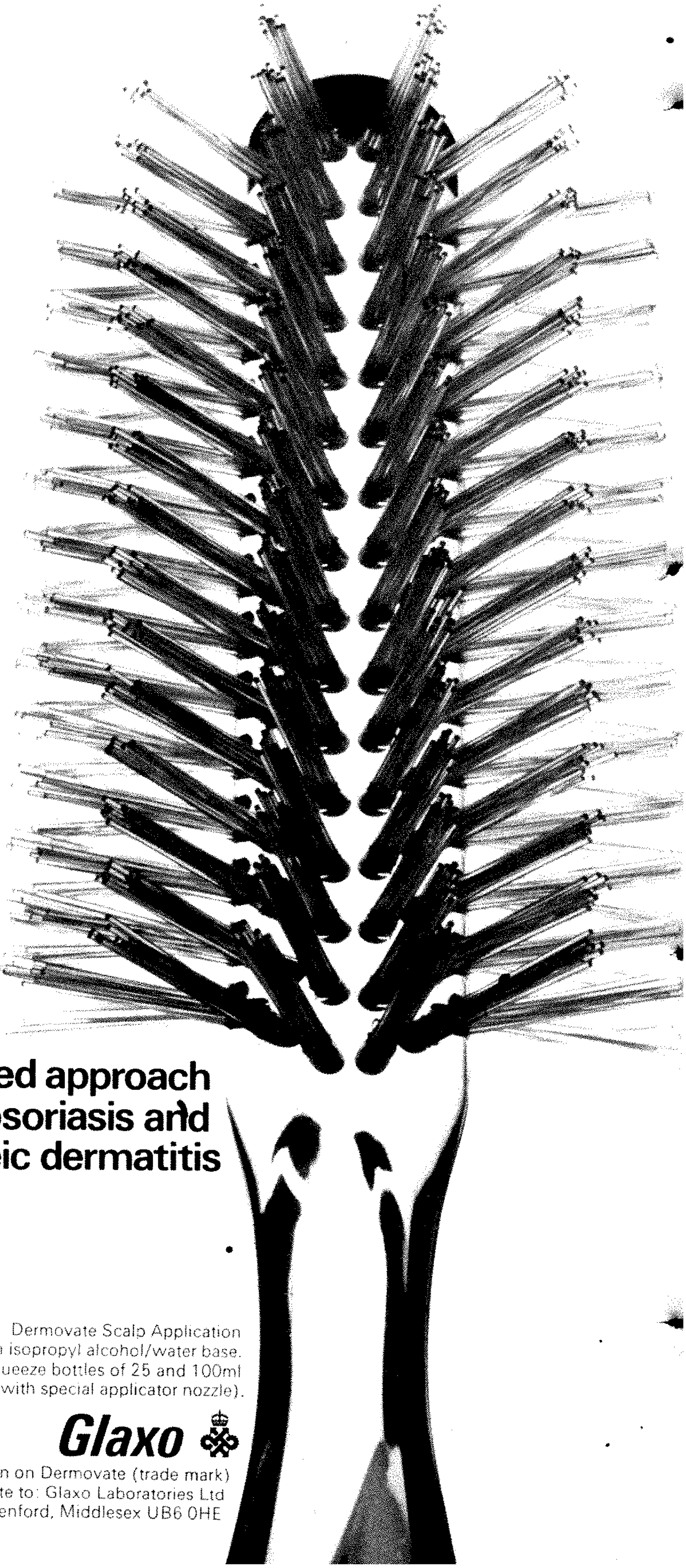

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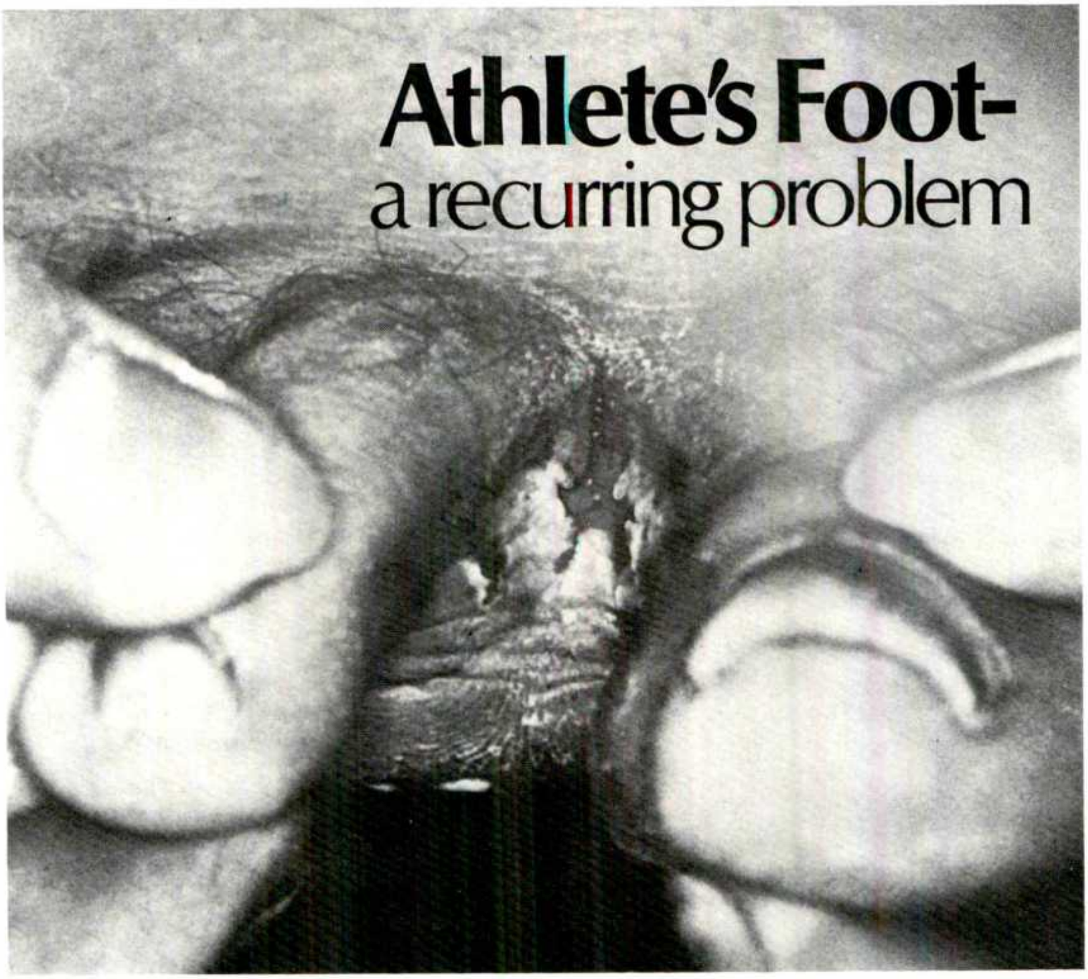

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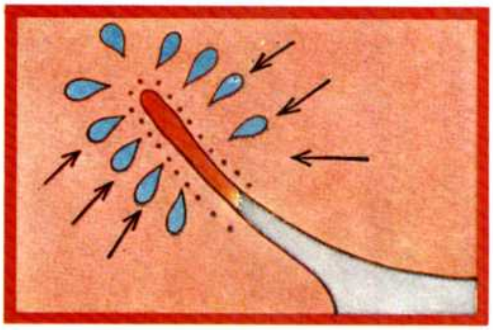

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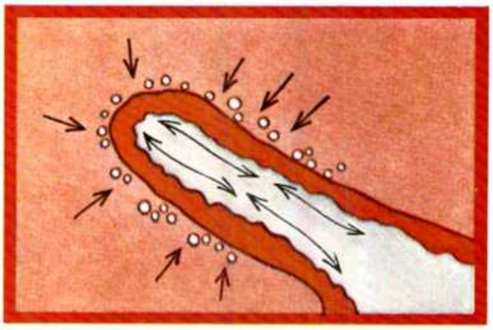

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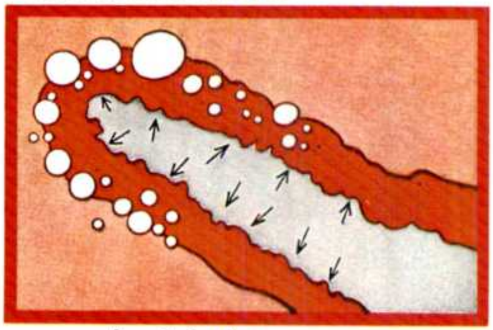

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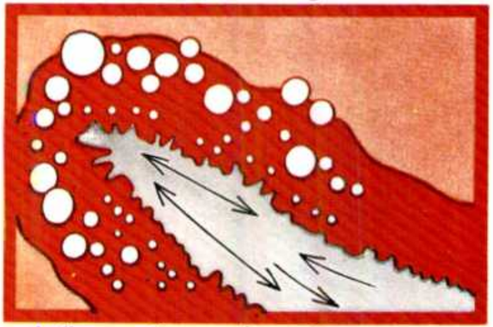

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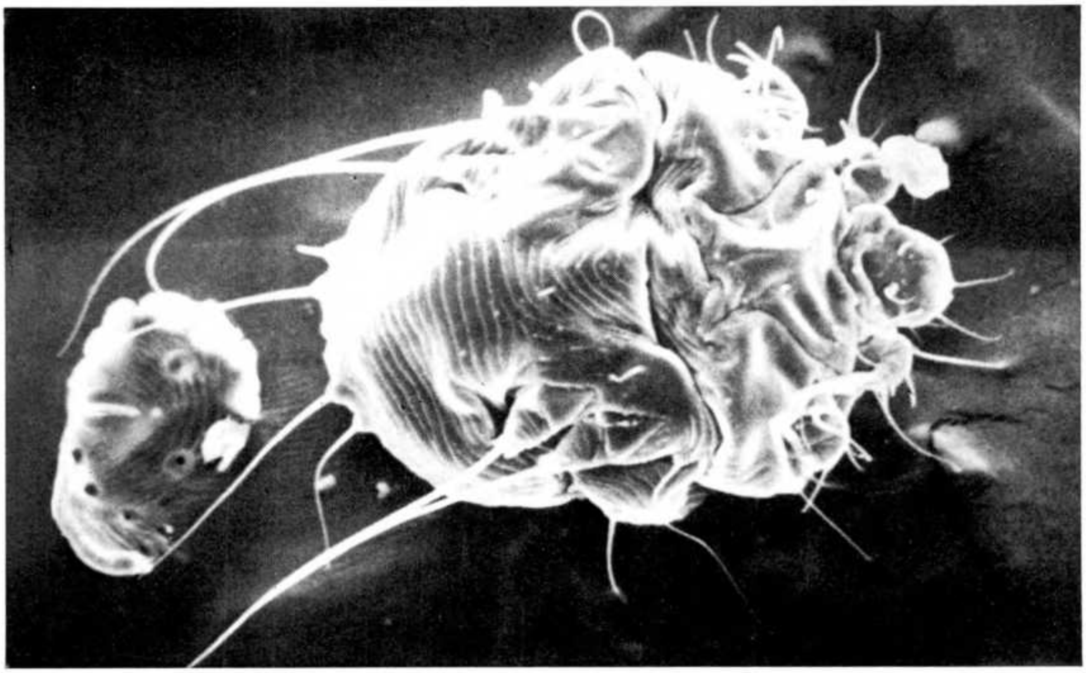

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Clinical and Laboratory Investigations

The premature ageing syndromes

REPORT OF EIGHT CASES AND DESCRIPTION OF A NEW ENTITY NAMED METAGERIA

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SUMMARY

Eight patients are reported who all have one feature in common, the appearance of premature ageing. Six of these could be accurately classified into previously described syndromes, but the other two may represent a new syndrome which we have called metageria. Clinical and other features of these two individuals are compared with three patients with acrogeria (Gottron's syndrome), one with pangeria (Werner's syndrome), one with progeria (Hutchinson–Gilford syndrome) and one with a total lipodystrophy (Lawrence's syndrome).

In 1886 Hutchinson described a boy who had been bald since infancy and whose skin was atrophic and wrinkled. This child and another patient were subsequently reported by Gilford (1904), who used the name progeria for the first time. In the same year, in his thesis entitled 'Cataract in combination with scleroderma', Werner (1904) gave a detailed description of four siblings with these signs together with greying hair and a senile appearance; this syndrome now usually bears his name. We suggest that pangeria might well be used as an alternative description for this disorder. Some years later, Gottron (1940) used the term acrogeria when he described two siblings with premature ageing of the skin, which was confined to the extremities and which had been present from birth.

The total lipodystrophies, where the absence of adipose tissue is often evident from birth, give affected children a wasted and prematurely aged appearance. Ziegler (1928) was the first to describe a patient with generalized lipo-atrophy. Subsequently other authors have recorded syndromes with total lipodystrophy, notably Lawrence (1946), whose patient developed the disease in her third decade of life, and Seip (1959), whose three patients had signs of the disease at birth. A comprehensive review by Senior & Gellis (1964) of the total lipodystrophies pointed out that not every feature was present in each patient, but the overall combination was usually distinctive.

It is clear from the descriptions that all these conditions have some characteristics in common, in particular they all give the appearance of premature ageing. We have recently had the opportunity to study eight patients in this group of disorders and six of these could be classified into previously described syndromes. The other two patients had several similar features, but could not be accurately diagnosed and may represent a new syndrome which we have called metageria.

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TABLE 1. Clinical features of the premature ageing syndromes

	Metageria	Pangeria (Werner's syndrome)	Acrogeria	Progeria	Total lipodystrophy
Stature	Tall and thin	Small stature. Cessation of growth at 12 years	Normal	Dwarf	90th percentile increased growth rate
Facies	Bird like facies, pinched face. Beaked nose	Beaked nose. Skin of ears atrophic and tightly bound down	Micrognathia. Atrophy of skin on tip of nose	Mid facial cyanosis. Bird like facies. Glyphic nasal tip. Prominent frontal tuberosities and scalp veins. Chin recessed	Pinched face with absent buccal pad of fat
Skin	Atrophic—most marked on limbs. Mottled hyperpigmentation. Telangiectasia	Dry atrophic skin. Mottled hyperpigmentation. Telangiectasia	Atrophic with telangiectasia and mottled hyperpigmentation on extremities	Dry, thin and wrinkled with mottled hyperpigmentation. May present with scleroderma-like changes on limbs	Coarse dry skin. Patchy hyperpigmentation. Hypertrichosis. Normal skin elasticity
Scalp hair	Fine and thin	Premature greying 20 years. Loss of hair 20–25 years	Normal	Hair lost in first 2 years of life	Abundant and curly
Eyes	Prominent eyes. No true exophthalmos	Bilateral juvenile cataracts (20–30 years). Keratopathy. Glaucoma	Normal	Prominent eyes, otherwise normal	Small punctate corneal opacities (Seip, 1959)
Nails	Normal	Normal	Dystrophic or thickened	Thin and brittle	Normal
Limbs	Generalized loss of subcutaneous fat. Ischaemic leg ulcers (one case)	Lower limb ulcers. Hyperkeratosis over bony prominences. Generalized loss of subcutaneous fat	Atrophy of skin most marked on extremities. No leg ulcers	Prominent joints. Coxa valga. Generalized subcutaneous fat loss. Poorly developed muscular system. No acrosclerosis or Raynaud's phenomenon	Apparent muscular hypertrophy due to loss of subcutaneous fat

PRESENT INVESTIGATION

Ascertainment

Six of the patients were referred to the genetic clinic at St John's Hospital for Diseases of the Skin. A widespread search for the condition has not been made. Where possible all living close relations in each kindred were seen and examined. Two patients (cases 1 and 2) have been seen at meetings of the Dermatology Section of the Royal Society of Medicine (Wells, 1972; Gilkes, 1972). Case 5 was reported by Calvert (1957) and case 7 by Moynahan (1962). All the patients had been under the care of other dermatologists before they were referred to us and we are grateful to them for their help.

Method of investigation

When they were first seen full pedigree details were recorded and a complete clinical examination was carried out. Six of the patients (cases 1–5 and 8) were admitted to Guy's Hospital for a short time so that extensive studies could be completed. Examination of dermatoglyphic patterns was reported by Dr J. Verbov, and Dr R.W. Baker was responsible for the lipid studies.

Classification

Two of the patients (cases 1 and 2) were diagnosed as metageria, three as acrogeria (cases 3, 4 and 5), and of the remaining individuals, one was diagnosed as pangeria (Werner's syndrome, case 6), one as progeria (case 7) and the last as a lipodystrophy (case 8).

The clinical and other features of these syndromes are summarized in Tables 1, 2 and 3.

TABLE 2. Clinical and endocrinological features of the premature ageing syndromes

	Metageria	Pangeria (Werner's syndrome)	Acrogeria	Progeria	Total lipodystrophy
Cardio-vascular system	Early athero-sclerosis. No vascular calcification.	Atherosclerosis. Vascular calcification. Coronary artery disease	Normal	Severe generalized atherosclerosis. Interstitial and focal myocardial fibrosis. Coronary artery disease	Cardiomegaly (Seip, 1959)
Skeletal	Normal	Osteoporosis. Soft tissue calcification	Normal	Cranio-facial disproportion. Peri-articular fibrosis. Hypoplastic mandible. Dystrophic short clavicles	Advanced bone age
Metabolic	Early onset diabetes mellitus	Mild mature onset diabetes mellitus at about 30 years of age	None	No consistent abnormality described	Hyperlipidaemia. Insulin resistant early onset diabetes mellitus. Hepatomegaly
Secondary sexual characteristics	Genitalia normal size. Normal libido. Pubic hair normal but sparse	Hypogonadism. Scanty pubic hair. Poor libido. Menopause early	Normal	Absence of sexual maturation	Pseudo-enlargement of genitalia

TABLE 3. Summary of mode of inheritance, sex incidence, age of onset and prognosis in the premature ageing syndromes

	Metageria	Pangeria (Werner's Syndrome)	Acrogeria	Progeria	Total lipodystrophy
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	?Autosomal recessive when genetically determined
Sex incidence	Both sexes affected	Both sexes affected	Most described cases female	Both sexes affected	Female: Male 2:1
Apparent age of onset	Changes present at birth	15–30 years	Changes present at birth	2nd month–1 year	Can be present at birth, often early adolescence
Prognosis	Dependent on severity of diabetes and atherosclerosis	Mean life span 47 years	Normal life	Usually die before 16 years old	Dependent on metabolic complications

CLINICAL FEATURES

Metageria: cases 1 and 2

Case 1. Female aged 24

She had always been abnormally tall and thin for her age with little or no subcutaneous fat anywhere on her body. When 12 years old, she developed areas of telangiectasia and mottled hyperpigmentation on her upper trunk and limbs. At this time she also developed stiffness and deformity of the fingers associated with Raynaud's phenomenon. Her leg became ulcerated when she was 7 years old and since that time she has had recurrent ulceration around both ankles and on her feet, associated with a very poor peripheral circulation. Bilateral lumbar sympathectomy only produced temporary relief and in the last 18 months she had developed peripheral gangrene of her toes and feet which had necessitated an amputation of her left foot and her right leg below the knee.

Endocrine features. Her periods began normally when she was 14 years old, but have since been irregular and scanty. At the age of 20 years she developed polyuria and polydipsia caused by diabetes mellitus which was controlled with chlorpropamide and phenformin.

On examination her height was 1·75 m and her weight was 31 kg. She looked older than her years because of her thin bird like facies, beaked nose and staring eyes, together with a virtual absence of all subcutaneous fat (Fig. 1). Her skin was atrophic and tightly bound down on the hands and feet so that the veins and tendons could be easily seen. There were multiple telangiectatic lesions scattered over the whole body but most numerous over the upper chest and limbs. Areas of mottled hyperpigmentation gave the skin a poikilodermatous appearance. Her scalp hair was of extremely fine texture with no evidence of greying. Her eyes were staring, but there was no true exophthalmos, nor was there an arcus senilis. Slit lamp examination showed no evidence of cataract formation, but on fundal examination there were a few hard exudates in relation to the temporal vessels.

Her blood pressure was within normal limits, and the peripheral pulses could be felt in both upper limbs but could not be felt below her femoral arteries in her legs. Before her amputations there were gangrenous changes in both feet and large ulcers on both feet and over the heels.

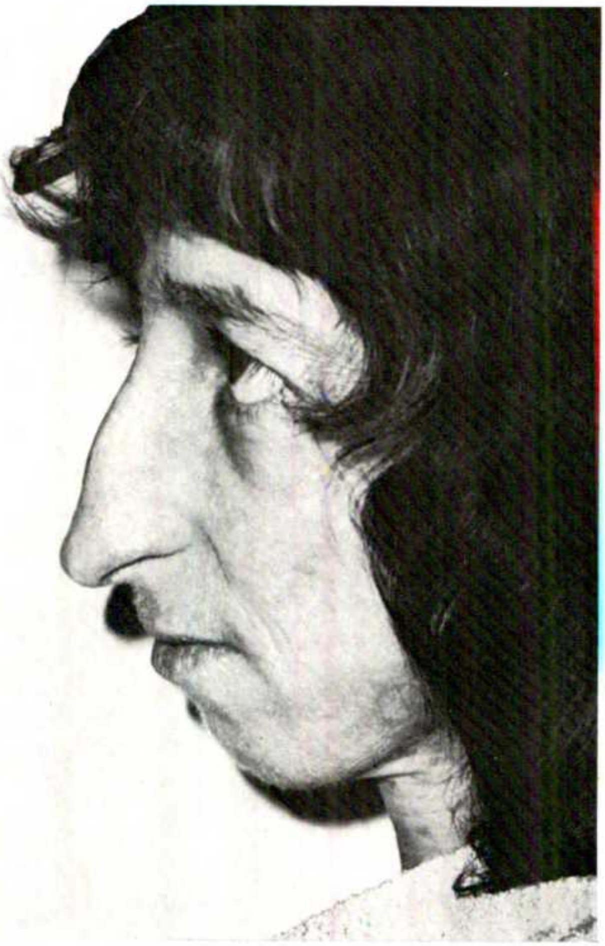

FIGURE 1. Metageria (case 1). Profile of face to show beaked nose and thin face.

Endocrine features. Her breasts were within normal limits and the thyroid gland was not palpable. The pubic and axillary hair was present but sparse and of fine texture.

This girl has since died aged 25 years and post mortem examination was not carried out.

Case 2. Male, aged 20 years

The almost total absence of subcutaneous fat anywhere on his body had been noted from infancy and he had always been tall and thin for his age. Hyperpigmented areas were first seen on the side of his neck when he was 10 years old, and subsequently his whole skin had a mottled hyperpigmented appearance. He has never had Raynaud's phenomenon or ulcers on his legs.

Endocrine features. Secondary sexual characteristics developed normally at puberty. At the age of 17 he developed polyuria and polydipsia caused by diabetes mellitus and this was controlled by soluble insulin 70 units daily.

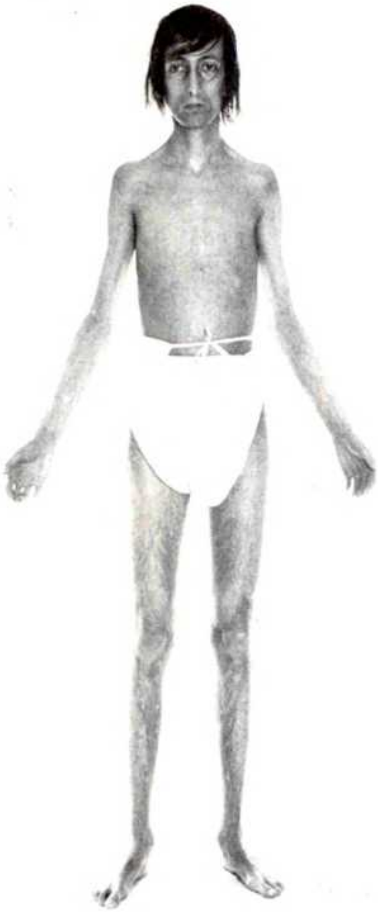

FIGURE 2. Metageria (case 2). Shows the general habitus and mottled pigmentation on the trunk.

On examination his height was 1·75 m and he weighed 43 kg (Fig. 2). The thin pinched face, with prominent eyes and beaked nose were very similar to Case 1 (Fig. 3).

His skin was soft and atrophic, in particular on the backs of his hands, so that the veins and tendons could be easily seen (Fig. 4). The mottled type of pigmentation was most marked over the shoulders and anterior iliac crest and although there was some telangiectasia it was not a prominent feature. His scalp hair was fair and of a rather straggly appearance and of extremely fine texture.

There was no arcus senilis, slit lamp examination showed no cataract and the fundi were normal. His peripheral pulses were normal but a grade II systolic ejection murmur, heard best down the left sternal edge, suggested aortic sclerosis. The aortic and pulmonary second sounds were normal.

His limbs were long and thin because of the virtual absence of subcutaneous fat. There was no apparent muscle hypertrophy and there was no leg ulceration.

Endocrine features. His genitalia were of normal size. The thyroid gland was not palpable. He had no

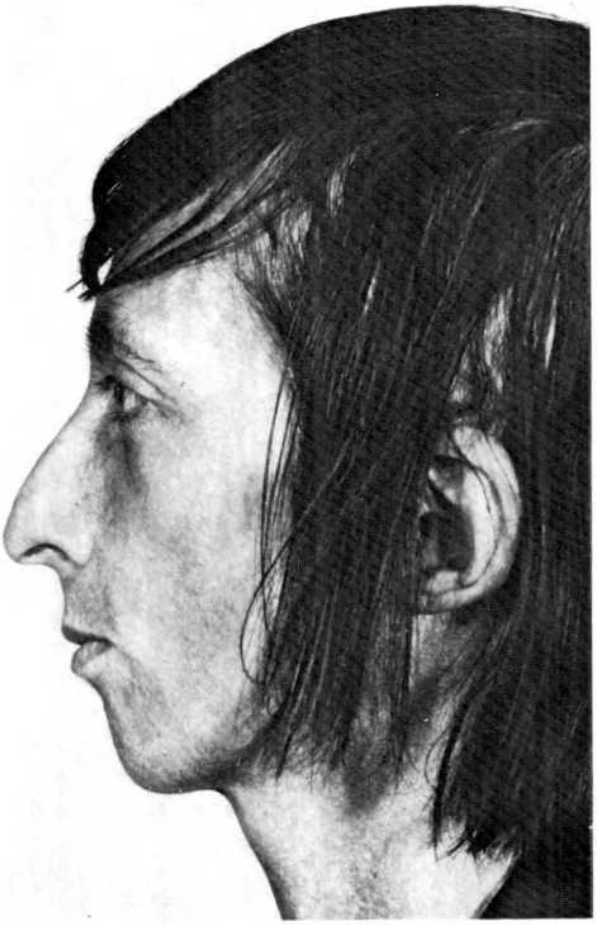

FIGURE 3. Metageria (case 2). Shows similar facial features to case 1.

axillary hair and although the pubic hair was very fine it was of a normal distribution. His voice had broken at puberty but was still moderately high pitched.

Summary (cases 1 and 2)

These two patients had a striking facial resemblance to each other, as well as several other features in common. They were of identical height with a similar body build and both patients had a virtual absence of subcutaneous fat. The atrophic and hyperpigmented skin was also very similar in each case, being most pronounced on the extremities. Development at puberty was normal in both patients and they had developed diabetes mellitus at a very similar age. The most obvious difference between them was the gangrenous change of one patient, but this was thought to be well within the differences which might be observed in a syndrome with some genetic variation.

Acrogeria: Cases 3, 4 and 5

Case 3. Female aged 22

This patient was born prematurely and her birth weight was 1·58 kg. She had always been noted to be

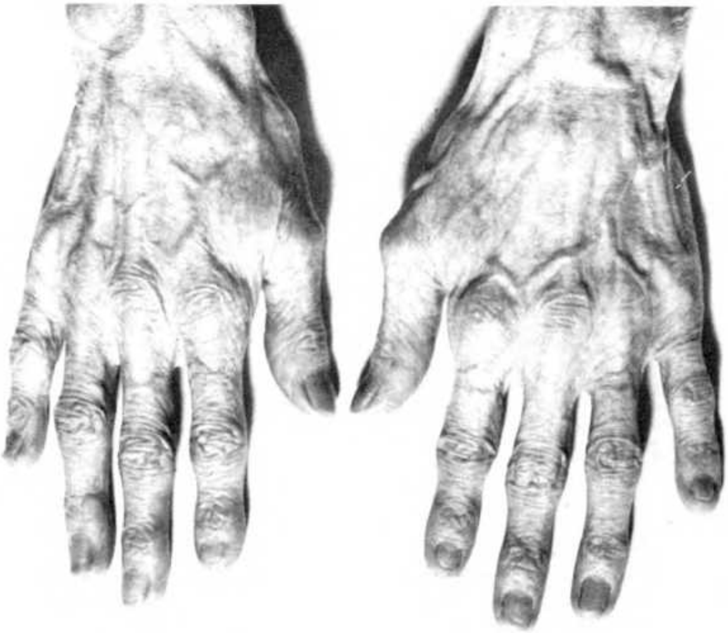

FIGURE 4. Metageria (case 2). Wrinkled atrophic skin over the dorsum of the hands with the veins and tendons showing prominently.

small and thin and the absence of subcutaneous fat was most obvious on her hands and feet. The skin on her extremities had always been atrophic and fragile with the result that she bruised very easily. She has never had Raynaud's phenomenon or ulceration of her limbs.

Endocrine features. Her periods began at the age of 10 and were regular and normal. She has never had diabetes mellitus.

On examination her height was 1·54 m and she weighed 41 kg. She was a small, thin girl with a generalized loss of subcutaneous tissue which was most pronounced on the extremities. She had a thin pinched face and pointed nose (Fig. 5). The skin on the backs of her hands (Fig. 6) and on the dorsa of her feet (Fig. 7) was wrinkled and atrophic. There was no increased pigmentation or telangiectasia, but the skin on her trunk was soft and atrophic with the veins showing through prominently. However, these changes were not nearly as marked as on her hands and feet.

The scalp hair was fine and rather frizzy, but there was no thinning or premature greying. Both eyes appeared prominent, but there was no true exophthalmos. Slit lamp examination was normal, but an arcus senilis was present in both eyes. She was normotensive and all the peripheral pulses were within normal limits, but there was a systolic ejection murmur in the aortic area which could also be heard down the left sternal edge. This suggested aortic sclerosis of the type that is usually found in much older people.

Endocrine features. Her breasts were small. The axillary and pubic hair was fine with a normal distribution.

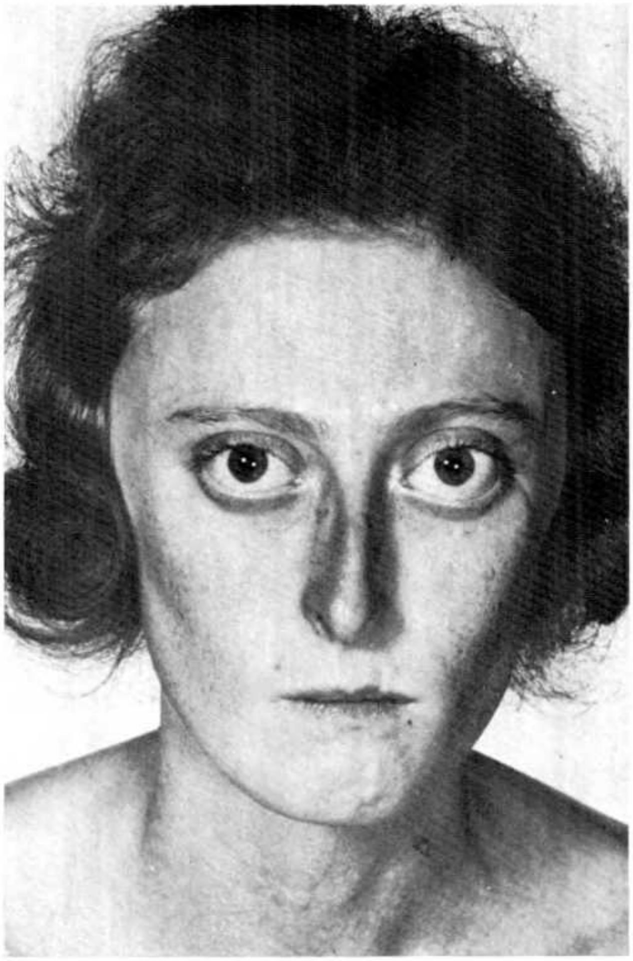

FIGURE 5. Acrogeria (case 3). Thin pinched face with prominent eyes.

Case 4. *Female aged* 18 *years*

She weighed 2·44 kg at birth and was always noted to be a small thin baby. Because of extensive bruising a tentative diagnosis of the Ehlers–Danlos syndrome was made when she was 2 years old. Eight years later she was noted to be on the third percentile for weight and the fortieth for height, with a marked loss of subcutaneous fat on the face and distal extremities.

The skin on her arms and legs has always been fragile and has needed suturing on many occasions. There has never been any ulceration on her limbs, but for the last few years she has had Raynaud's phenomenon.

Endocrine features. Her menses have been extremely heavy since the menarche when she was 14 years old. Subsequently these have been controlled with ethinyl oestradiol. She has never had diabetes mellitus.

On examination her height was 1·54 m and she weighed 40 kg. She was a small thin girl with a

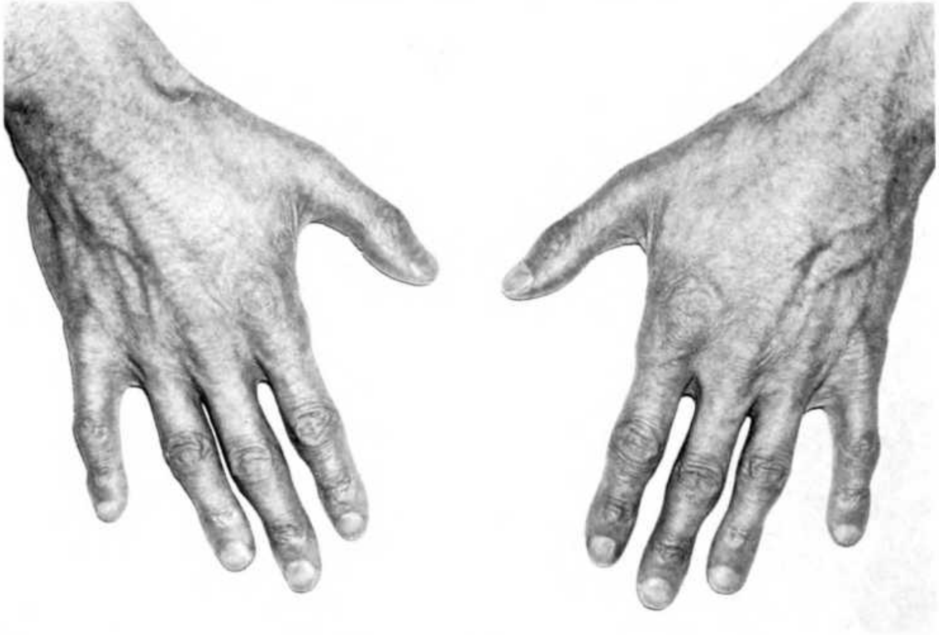

FIGURE 6. Acrogeria (case 3). The skin over the dorsa of the hands with a premature aged appearance similar to case 2 (Fig. 4).

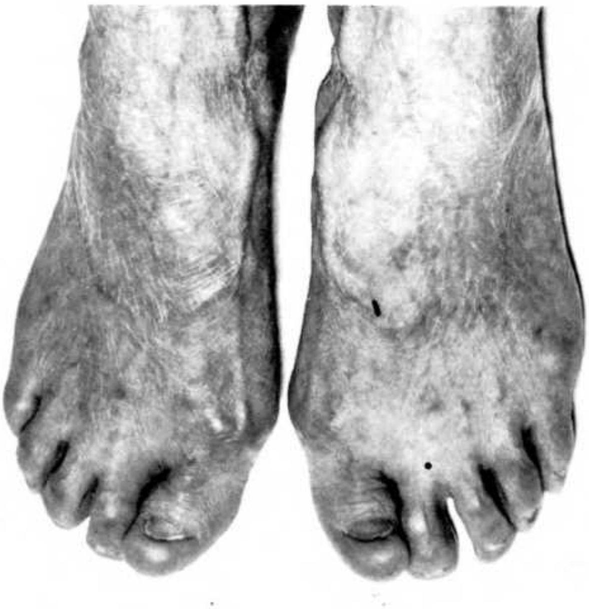

FIGURE 7. Acrogeria (case 3). Atrophic skin on the feet.

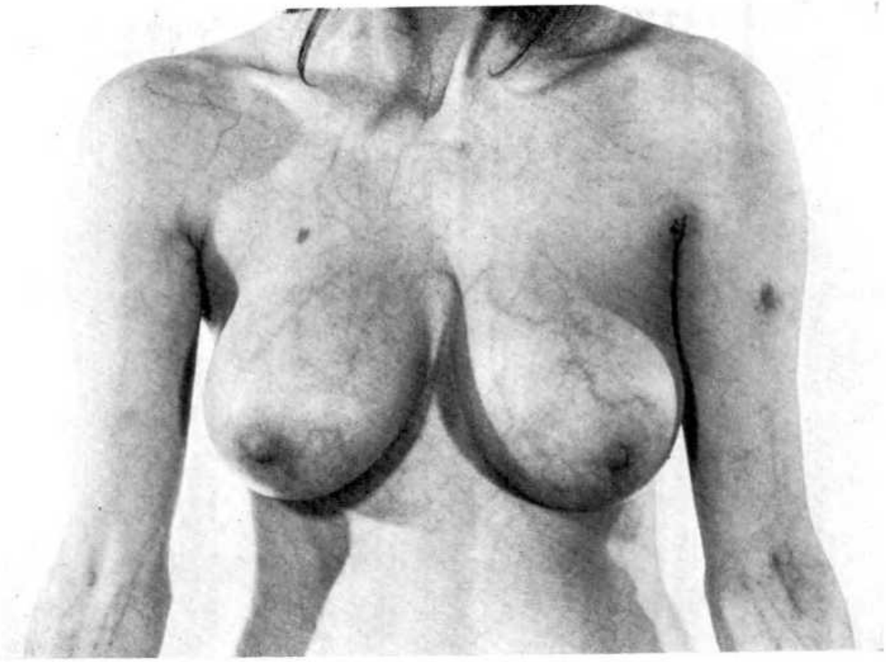

FIGURE 8. Acrogeria (case 4). Showing atrophic skin on the trunk through which the veins can be easily seen.

generalized loss of subcutaneous tissue, which was most noticeable on the hands and feet. Her face was pinched with a small pointed nose and moderate micrognathia. The skin on her limbs was soft and atrophic, but was not abnormally bound down to deeper structures. The veins and tendons stood out prominently on the backs of her hands and the dorsa of her feet, a feature which made them look senile. There was no hair on her hands or feet. There were large, poorly healed scars on both knees. The skin on her trunk was also abnormal, but to a lesser degree, and the veins showed through prominently (Fig. 8). There was no telangiectasia on the trunk but there was some livedo patterning on her lower legs.

Her scalp hair was brown and there was no evidence of thinning or premature greying. She had a bilateral arcus senilis in the lower half of both eyes. Her blood pressure was normal. A systolic ejection murmur was heard down the left sternal edge and this, together with a closely split aortic second sound with a normal carotid pulse, suggested aortic sclerosis without obstruction.

Endocrine features. Her breasts were normal with prominent veins. The axillary and pubic hair was fine and scanty.

Case 5. Female aged 22 years

This patient was born prematurely and weighed 0·91 kg at birth. From that time her hands and feet have been noted to be small with atrophic, wrinkled skin making them look as if they belonged to an older person. The rest of her body had always been thin, but not to the same extent as her hands and feet.

She bruised with only minor trauma and the soft atrophic skin was easily lacerated and constantly needed suturing. Her hands and feet were often extremely cold in the winter, but she did not have a true Raynaud's phenomenon. She has never had any ulceration of the lower limbs.

Endocrine features. Her periods began at the age of 11 and had always been normal. She has never had diabetes mellitus.

On examination she was a small, thin girl whose height was 1·54 m and weight 38 kg. There was a generalized loss of subcutaneous tissue, most marked on her hands and feet. Her face was thin and pinched with hollow cheeks and a small pointed nose. The skin on her hands and feet was soft and atrophic and looked senile. These changes on the extremities were more marked than on the trunk, but the skin was atrophic and the veins stood out prominently on her body.

Her scalp hair appeared to be normal and there was no thinning or premature greying. Her eyes looked normal and slit lamp examination was also normal. The cardiovascular system showed no abnormality.

Endocrine features. Her breasts, pubic and axillary hair were normal.

Summary (*cases* 3, 4 *and* 5)

These three patients were all noted at or soon after birth to have an abnormality of their skin which was most marked on the extremities. The skin of the hands and feet was atrophic, wrinkled and bruised easily. The pinched facies with moderate micrognathia were also a feature of the two siblings described by Gottron (1940). The life expectancy is said to be normal in the patients with acrogeria, and apparent premature ageing of organs other than the skin has not previously been reported. The evidence of aortic sclerosis and arcus senilis in cases 3 and 4 may indicate that the ageing process is more widespread than was once thought. No metabolic or endocrine abnormalities have been described in this condition and none was found in our three patients.

Pangeria (*Werner's syndrome*)

Case 6. *Male aged* 43 *years*

This patient had no apparent abnormality until at the age of 28 years he developed leg ulcers which were unresponsive to treatment. As a boy he had fine fair hair, but this became grey at the age of 30 years and has since been lost predominantly on the temples and crown. He developed bilateral cataracts, which necessitated lens extraction from both eyes, at the age of 33 years.

Endocrine features. Facial and pubic hair developed at the normal age and he says he has to shave daily. Libido is reduced and he is unmarried. His voice remains high pitched and he has often been mistaken for a woman on the telephone. He has never had any evidence of diabetes mellitus and tests for this condition have been carried out on many occasions.

On examination he was a small man whose height was 1·62 m and weight 50 kg. He looked very much older than 43 years because he had thin wizened features and a small pointed nose. The skin over most of his body was atrophic with some patchy hyperpigmentation on the lower limbs, but no telangiectasia. His scalp hair was fine and grey with considerable balding.

The lenses had been removed from both eyes. His left fundus was obscured by an old haemorrhage and the right fundus showed a few soft exudates. The cardiovascular system was normal except for absent pulses below the knees of both lower limbs. There was extensive ulceration around both ankles.

Endocrine features. His genitalia were small. The pubic and axillary hair were fine and sparse with a normal distribution.

Comment

This patient had all the features of pangeria (Werner's syndrome), as listed by Thannhauser (1945), with the exception of diabetes mellitus. Diabetes was only a feature in half the patients with pangeria reviewed by Epstein *et al.* (1966) and was usually of the mild, mature onset, form.

Progeria (Hutchinson–Gilford syndrome)

Case 7. *Male aged* 12 *years*

This boy's skin was first noted to be thin and atrophic at the age of 1 year. There was apparent absence of subcutaneous fat and his skin appeared bound down to deeper structures. When aged 18 months he lost most of his scalp hair and was bald by the age of 2 years. At that time he had the classical appearance of progeria.

Endocrine features. Secondary sexual characteristics had not developed.

On examination he had the appearance of a wizened old man whose height was 0·94 m and weight 11 kg. He had the typical bird-like facies, with prominent frontal bones and scalp veins, and large bat-ears.

His skin was wrinkled and atrophic with some patchy pigmentation on his trunk. There was absence of scalp and body hair. Although his eyes appeared prominent they were otherwise normal. He had a marked tachycardia and evidence of left ventricular hypertrophy and strain. There was swelling of the interphalangeal joints of his hands and also of the knee joints. The movement of all his joints was limited. His clavicles were thin and there was a marked dorsal kyphosis. He had a koilonychia of some finger nails.

Endocrine features. His genitals were those of a prepubertal boy and there was no pubic hair.

This boy has since died aged 13 years and post mortem examination was not carried out.

Comment

The difficulty in diagnosing progeria arises in the first 2 years of life. After this period the classical features are usually obvious.

Lipodystrophy

Case 8. *Female, aged* 29 *years*

This patient was always noted to be very thin, until the age of 12 when there was a great increase in the amount of subcutaneous fat on her abdomen. However, the rest of her body remained extremely thin. Her skin was soft, but there was no atrophy, hyperpigmentation or telangiectasia.

The fat on her abdominal wall increased to such an extent that she has had to have an apronectomy on three occasions. During one admission for this operation she was found to have diabetes mellitus for which she needed insulin. At first this was controlled on relatively low dosage, but by the age of 20-years she needed 300 units of soluble insulin daily. To overcome the insulin resistance she had been given systemic steroids for the last 2 years. During this period she developed extensive eruptive xanthomata on her limbs.

Endocrine features. Her periods began normally when she was 14 years old, but have become irregular with a heavy loss. She has not been pregnant. Her diabetes mellitus has remained difficult to control and she is still on a large dose of insulin.

On examination she was a small woman whose height was 1·57 m and weight 50·8 kg. She looked very much older than her true age because of her thin, pinched bird-like facies and pointed nose.

Her skin was soft but there was no atrophy or telangiectasia. Numerous smooth xanthomata were present on the upper arms and thighs with a few scattered lesions on the trunk. The scalp hair was fine and fair and there was no sign of thinning or greying.

Her eyes looked prominent because of her thin face, and slit lamp examination was normal. Some haemorrhages and hard exudates were seen on both retinae. Her abdomen was still very large and scarred from the apronectomies, and her liver was enlarged and palpable two finger-breadths below the right costal margin.

Endocrine features. Her breasts, axillary and pubic hair were all within normal limits.

Comment

The marked hyperlipidaemia (Table 4), insulin resistant diabetes mellitus, hepatomegaly and abdominal protruberance together with the total absence of subcutaneous fat on her limbs are all features of a total lipodystrophy. In adults with total lipodystrophy increase in height is not a feature and muscularity is less obvious (Senior & Gellis, 1964). This case history has been included as the facies and appearance of the limbs are similar to the previous conditions described with premature ageing.

LIPID STUDIES

Extensive lipid studies were carried out on the fasting serum of all the patients except the boy with progeria. The serum lipids measured were cholesterol, phospholipid phosphorous, triglycerides, the individual fatty acids, thin layer chromatography and electrophoresis on cellulose acetate and polyacrylamide gel. The results of these investigations are summarized in Table 4.

Although there were slight abnormalities in lipo-protein bands on electrophoretic examination in some of the patients, there was no consistent abnormal pattern for either the patients with metageria or acrogeria. Striking abnormalities were seen in the female with a lipodystrophy (case 8). She had a lipaemic serum and high lipid values.

The results suggested that an abnormality of lipid metabolism is unlikely to be found in individuals with either metageria or acrogeria.

OTHER INVESTIGATIONS

Haematology and biochemistry

The six patients who were admitted to hospital had very extensive investigations and the other two had similar studies carried out while attending out-patient clinics. These included estimations of haemoglobin, white cell count, ESR, serum iron, plasma proteins and electrophoresis; liver function tests, thyroid function tests and other appropriate investigations. These were normal except in case 1 who had a haemoglobin of 10 g% and an average ESR of 45 mm/h. A glucose tolerance test was done on two of the non-diabetic individuals and the patient with pangeria (Werner's syndrome) had a recent normal test.

Radiographic studies

All eight patients had normal chest radiographs and five patients (cases 1–5) had normal radiographs

TABLE 4. Summary of lipid studies

	Metageria		Acrogeria			Pangeria	Lipo-dystrophy
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 8
Phospholipid phosphorus μmol/ml (normal 2·5–3·2)	2·7	2·4	2·7	2·2	2·3	3·2	6·4
Cholesterol mg/100 ml (normal 120–230)	170	160	230	160	165	227	497
Triglycerides mg triolein/100 ml (normal 100–300)*	150	190	158	119	104	278	1368
Total fatty acids μmol/ml (normal 12–14·5)	13·8	12·4	14·2	7·9	12·2	17·6	93·8
Electrophoresis							
Oil red stain for lipids on cellulose acetate	α_1(+) δ Slow β	Normal	Normal	Slow β	Slow β	Normal	Raised but no abnormal pattern
Ponceau red stain for proteins on cellulose acetate	α_1 (+) α_2 (+) γ (−)	Albumin (−)	Albumin (−) γ (−)	α_1 (+)	α_1 (+)	Alb. (+) α (+) β (+) γ (+)	Pre-alb (+) α_1 (+) α_2 (++) γ (+)
Amido—Schwartz stain for proteins on polyacrylamide gels	Band below β very high Band above β slightly raised	Band below β raised	Normal	Band above β rather slow	Haptoglobin bands distinct and slightly raised	Normal	Normal

*For males; females 70–90 mg triolein/100 ml (R.W.Baker—unpublished results).

of their limbs. A translumbar aortogram (case 1) was reported as follows: "With the exception of the renal arteries, she has a 'micro-arteria' affecting the abdominal aorta and all vessels distal to this. The arteries appear to be unusually smooth with the exception of one or two localized areas, where there appears to be narrowing due to plaque formation. This was not considered to be the classical appearance of arteriosclerosis."

Electrocardiograms

Apart from cases 5 and 6, all the patients had an electrocardiogram and in summary the results suggested ischaemic changes which would usually be found in much older people.

Histology

Skin biopsies from the forearm of cases 1 and 2 showed an atrophic epidermis with loss of rete pegs, acellular collagen in the upper dermis and the appendages were reduced in size. There was a normal subcutaneous adipose layer. By contrast, patients with a total lipodystrophy have a subcutaneous layer replaced by acellular fibrous tissue (Fairney, Lewis & Cottom, 1969).

DERMATOGLYPHICS

Dermatoglyphic studies were recorded by Dr Julian Verbov on patients 1, 2 (metageria) and 3 (acrogeria). He noted:

Case 1. The skin over the hands was atrophic and the main palmar creases were not clearly visible on examination. Fixed flexion deformity at the proximal interphalangeal joint of some fingers made printing of the distal palms and fingers difficult. Definite ridges were visible over the proximal palm, and, with magnification, ulnar loop patterns could be defined over the eight fingertips that could be seen reasonably clearly. No patterns were visible in either hypothenar or thenar/first interdigital areas. The axial triradius was in the normal position on both palms. Plastic imprint of an area in the left hypothenar region showed marked ridge flattening, but pores were clearly visible in some areas, though not in sufficient numbers to give a realistic count. Sweat testing of the right hypothenar area using *o*-phthaldialdehyde was normal.

Case 2. The skin was atrophic over the hands; there was hyperkeratosis over the plantar aspect of the heels and the balls of the feet. Finger prints were clear on most digits: digits V (little finger) on the right showed a radial loop which is an uncommon finding on this digit. Patterns were absent in thenar/first interdigital areas but a whorl was visible in the right hypothenar area. Plastic imprint of an area in the left hypothenar region (near the distal transverse crease) was normal. Sweat testing over the right hypothenar area with *o*-phthaldialdehyde was normal.

Case 3. Skin atrophy was very marked over both hands and feet. Ridges over the thumbs and index tips showed some 'breaking up' on finger prints, particularly the left thumb, but this was probably not a true ridge dissociation. Ridge distortion was clearly seen over the soles on printing. Neither hypothenar nor thenar/first interdigital patterns were present and axial triradii were in the normal position.

Plastic imprint of an area in the left hypothenar region (near the distal transverse crease) showed some ridge flattening but a normal pore count. Sweat testing in the right hypothenar area was normal.

Summary

There was minor ridge flattening and distortion, but no significant consistent abnormality. Sweat testing with *o*-phthaldialdehyde was normal in each case.

GENETIC FINDINGS

Both the patients with metageria had unaffected parents and siblings and there was no consanguinity. The three individuals with acrogeria all had unaffected parents and siblings and consanguinity was unlikely in all three families. Case 5 had an unaffected child. The father of the patient with pangeria (Werner's syndrome) derived from Poland and his mother's family were English. His two siblings were normal. Pedigree studies were unhelpful in the patient with progeria. The father of the woman who had a lipodystrophy died of a coronary thrombosis at the age of 58 and her mother also died of a coronary thrombosis at the age of 50. She had three younger siblings.

There was no family history of diabetes mellitus, other than in the patient with pangeria (Werner's syndrome). Both his maternal grandparents developed diabetes mellitus of late onset.

Comment

Pedigree studies in this group of patients did not give any help in determining the mode of inheritance of these disorders, because they were all sporadic cases. However, because both sexes were known to be affected and the diseases were both rare and severe, inheritance as an autosomal recessive trait might be postulated for all these conditions. There is some pedigree evidence in the literature to support this hypothesis in most of them.

In his original report, Gottron (1940) described siblings who were both affected with acrogeria. Similarly, Werner (1904) reported affected siblings, and Epstein *et al.* (1966) in their review of the literature found a high degree of consanguinity and that all the published pedigrees were compatible with an autosomal recessive mode of inheritance. In his long review of the Hutchinson–Gilford progeria syndrome, DeBusk (1972) found three reports of families where siblings were affected with the syndrome. In one family there was consanguinity. However, he thought it was more likely that the affected individuals were recipients of a new abnormal autosomal dominant mutation, although there was very little evidence to support this hypothesis. The genetic aspects of the genetically determined lipodystrophies have not yet been elucidated beyond doubt, but again an autosomal recessive mode of inheritance is likely. Although our two patients with metageria may be the recipients of a new autosomal dominant mutation, we consider the disorder is more likely to be inherited as an autosomal recessive trait.

DISCUSSION

Little is known about the origins of senescence and little agreement exists regarding the true nature of the ageing process (Goldstein, 1971). However, most authors would probably agree that greying of the hair, wrinkling of the skin and atherosclerosis are associated with the ageing process. The eight patients described in this report all had some features of ageing, which were observed long before they might have been expected.

Several syndromes have been described in which there is evidence of premature ageing; in historical order they are progeria, pangeria (Werner's syndrome) and acrogeria. Individuals with a total lipodystrophy are often included in this group, because they have an emaciated senile appearance due to the absence of subcutaneous fat. To these we should add metageria, because the two patients that we describe have sufficiently distinctive clinical features to differentiate them from the other groups.

Progeria

The patients described by Gilford (1904) and others were apparently normal at birth, although three features presented early in life; midfacial cyanosis, skin resembling scleroderma and a sculptured and grooved tip of the nose (De Busk, 1972). The diagnosis was easier in the second year of life, when hair was shed from the scalp and body, and the facies developed characteristically with prominent frontal tuberosities and easily visible scalp veins. The failure to grow or gain weight was usually observed in the first year of life. The skin became dry and wrinkled with a mottled pigmentation and the nails thin and brittle. There was a generalized loss of subcutaneous fat apart from the cheeks and inguinal regions. Peri-articular fibrosis caused stiffness and prominence of most of the joints with subsequent partial flexure of some joints which gave rise to a characteristic 'jockey' stance. Ossification dates were usually normal, but thinned cortices and poorly defined trabeculae gave a characteristic gracile appearance of the long bones. The dentition was often delayed and the mandible hypoplastic. Patients showed no signs of puberty at the usual age. However, the motor and mental development were normal.

The coronary and cerebral arteries were affected as part of the generalized atherosclerosis. Interstitial and focal myocardial fibrosis were also present (Reichel & Garcia-Bunuel, 1970). Very few affected individuals survived beyond their sixteenth birthday, because of the extensive myocardial changes (Atkins, 1954).

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Pangeria (Werner's syndrome)

The four siblings described by Werner (1904) had very similar clinical findings, namely shortness of stature, a senile appearance, greying of the hair beginning at the age of 20 years, cataracts appearing during the third decade, skin changes which he called scleroderma and an early cessation of menstruation.

Thannhauser (1945), in a comprehensive study of this syndrome, was the first to differentiate the skin changes from those seen with scleroderma and also from those observed in Rothmund's syndrome (1868). In addition to the characteristics originally described by Werner (1904), he added: premature baldness; trophic ulcers of the legs; hypogonadism; a tendency to diabetes mellitus; calcification of blood vessels; metastatic calcifications and osteoporosis.

Epstein *et al.* (1966) reviewed 125 cases of Werner's syndrome. Most of the patients were of short stature, because they stopped growing at about the age of 13 years. They had a characteristic habitus with disproportionately thin limbs compared with a moderately stocky trunk. Secondary sexual characteristics were poorly developed with hypogonadism and a decreased libido. Fertility was generally considered to be low, but nineteen of their female patients had a total of fifty-three pregnancies. Diabetes mellitus occurred in only half the patients and was usually of the late onset type and controlled by diet.

The principal areas of skin involvement were the face and distal extremities. The facies were described as 'bird-like' with a beaked nose and tight atrophic skin on the ears. The skin on the trunk felt dry and was atrophic with some hyperpigmentation and telangiectasia. Hyperkeratoses occurred over bony prominences such as the malleoli and these areas often ulcerated. Hair changes generally occurred at the end of the second decade of life, when it became grey and fell out, most commonly from the scalp, eye brows and eye lashes. In males, facial as well as body hair usually decreased.

The eyes occasionally appeared to be prominent because of loss of peri-orbital tissue. Ocular complications occurred very commonly at the end of the third decade of life in the form of cataracts. These were posterior cortical or subcapsular opacities, generally striate or homogeneous in nature and usually bilateral. A keratopathy and other corneal changes as well as glaucoma have often been recorded. Many patients showed some evidence of coronary artery disease as indicated by an abnormal ECG, myocardial infarction or congestive cardiac failure. On radiological examination, vascular calcification of the Monckeberg type was seen in about one-third of the patients, generally in the tendons and ligaments of the knees, ankles and elbows. Patchy or generalized osteoporosis was seen in about half the patients with pangeria (Werner's syndrome).

From these descriptions it will be seen that the changes in this syndrome are very characteristic and the diagnosis should not present any difficulty in patients over 20 years of age. However, the syndrome is very seldom diagnosed before this age and practically never before puberty. This is an important point which serves to distinguish patients with this condition from those with other syndromes. Although the term has not been used before, we suggest that the name pangeria might be used for these individuals, rather than the non-descriptive eponym.

Acrogeria

The clinical descriptions of acrogeria given by Gottron (1940), Gromzig (1951) and Gruneberg (1960) corresponded very well with the features described in our three patients. They said the skin on the hands and feet was atrophic, dry and wrinkled and that these changes extended about 5 cm up the limbs. There was mottled pigmentation and telangiectasia on the upper trunk, together with some softness and atrophy of the skin so that the veins showed through very prominently (Bazex & Dupré, 1955). Micrognathia was a feature of the original two siblings described by Gottron (1940). These individuals do not have an increased susceptibility to diabetes mellitus and an associated metabolic abnormality has not been described. The life expectancy is said to be normal.

Although the changes are generalized, they are more obvious in the first instance on the hands and feet and therefore the term acrogeria does give some indication of the clinical condition. Although the changes are present from birth or shortly after birth, it is unusual for the syndrome to be diagnosed in the first 2 years of life, but it can be diagnosed well before puberty. Our three patients all had a very low birth weight and remained small.

Lipodystrophy

Although patients with this condition can be readily diagnosed by their abnormal lipids, one individual was included in this series because of the very similar clinical features. Her skin showed no atrophic changes, but the lack of subcutaneous fat on her limbs and the pinched facies gave her an aged and wizened appearance.

When Senior & Gellis (1964) reviewed the reported cases of total lipodystrophy, they summarized the main clinical features that were usually present, but stated that not all the features had to be present in one patient. In children there was increased stature associated with an increased growth rate and an advanced bone age. Increased skin pigmentation, hypertrichosis and muscular prominence were also features. Abdominal protuberance and hepatomegaly were often apparent, together with some genital enlargement. When they were investigated these patients usually had a hyperlipidaemia. Diabetes mellitus generally developed later in life, even if it was not present in childhood.

Although signs of this disease can be present at birth (Seip & Trygstad, 1963), there are many features by which it can be distinguished from acrogeria.

Metageria

From the descriptions in the literature and the details given of our own patients, it will be apparent that the first two individuals cannot be classified into any of these four groups. Children with progeria have a characteristic appearance and die young. Our patients with metageria were noted to be abnormal early in childhood, but the changes are not localized to the hands and feet in the first instance, they were tall and they both developed diabetes mellitus. By contrast with Werner's syndrome the changes were noted too early, they were too tall and had normal secondary sex characteristics and their diabetes mellitus was of early onset. Although it is possible that these two patients may develop grey hair and cataracts before the age of 30 years, there was no evidence of either of these changes when they were seen and certainly there was no loss of hair.

When the two patients with metageria were in adjacent wards, despite some differences their appearance was so strikingly similar that they were often thought to be siblings. Both their faces were pinched with a beaked nose, the skin of both was dry and atrophic and both had mottled pigmentary changes with some telangiectasia. Although these syndromes have many clinical features in common, we concluded that these two patients had a separate disorder. At the present time it cannot be stated if the syndrome is genetically determined.

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The papulonecrotic tuberculide

FROM ARTHUS REACTION TO LUPUS VULGARIS

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SUMMARY

Ninety-one cases of papulonecrotic tuberculide were seen by the Department of Dermatology during the past 17 years. A focus of tuberculosis was found in 38%. Associated clinical phenomena included tuberculous arteritis with gangrene and the development of lupus vulgaris from papulonecrotic tuberculides. It is suggested that these tuberculides begin as an Arthus reaction which is followed by a delayed hypersensitivity response. The latter will prevent local proliferation of *Mycobacterium tuberculosis* in most cases, but in rare cases local multiplication of the organisms may lead to the development of lupus vulgaris. All patients improved rapidly on antituberculous treatment.

Genuine cases of papulonecrotic tuberculide are becoming scarce in developed communities. There is an inclination to doubt both the existence and the tuberculous origin of these lesions (Flegel, 1962; Lever, 1967; Wolff, 1971). The sharp decline in tuberculosis in these communities may account for this scepticism. In 1900 tuberculosis was one of the three leading causes of death in the United States, but by 1970 it no longer featured as one of the first ten (Dingle, 1973). The incidence of tuberculosis in the African is at present comparable with that in Whites a century ago in Europe. In the Pretoria district the case rate is fifteen times higher in the African than in Whites (300 and nineteen cases respectively per 100,000). The death rate is twelve times higher in the African than in Whites (sixty and five cases respectively per 100,000) (Pretoria Medical Officer of Health, 1971). It is, therefore, hardly surprising that we still see tuberculosis in the African much as the dermatologists must have done in Europe at the beginning of the present century.

PAPULONECROTIC TUBERCULIDES

A diagnosis of papulonecrotic tuberculide was made ninety-one times in the past 17 years—in eighty-six Africans and five Whites. The affected sites in order of frequency were the legs, knees, elbows, ears, face, buttocks, trunk and perineum in both sexes. In a few instances the glans and shaft of the penis were affected. Phlyctenular conjunctivitis occurred in 16%. Two-thirds of the cases occurred before the age of 30 years (Table 1). In about one-third of the cases a deep focus, most commonly a cervical adenopathy, some with scrofuloderma, was demonstrated (Table 2). It was striking that the

TABLE 1. Age distribution of onset of papulonecrotic tuberculides in ninety-one cases

Age (years)	> 10	11–20	21–30	31–40	41–50	51–60
% of cases	26	21	30	4	13	6

TABLE 2. Localization of the tuberculous focus in ninety-one cases of papulonecrotic tuberculide

Lymph glands	Lungs	Vertebrae and joints	Urogenital	Total
19%	12%	4%	3%	38%

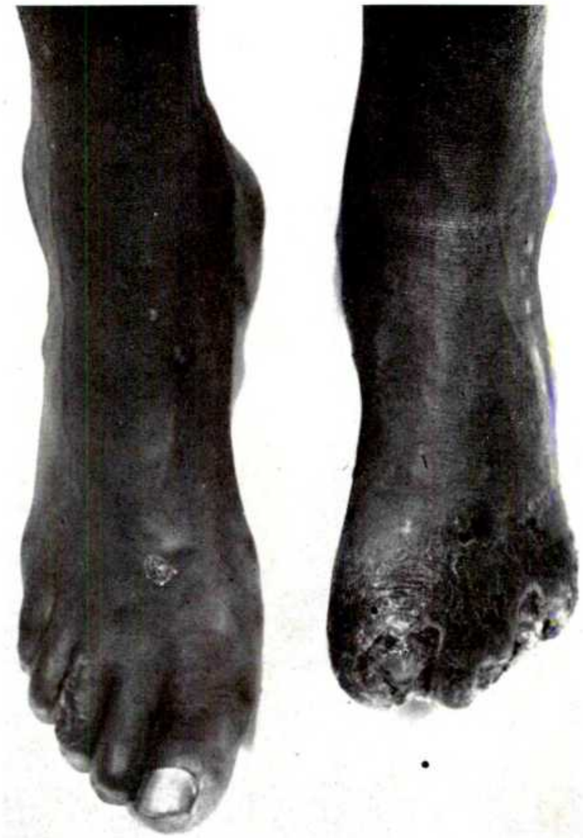

FIGURE 1. Gangrene of the left foot with papulonecrotic tuberculides on both feet. This patient also had widespread papulonecrotic tuberculides, lupus vulgaris on the shoulder and pulmonary tuberculosis.

eruption of new tuberculides stopped and an improvement of existing lesions followed within a few days of treatment with streptomycin and isoniazid, whether a deep tuberculous focus was known to exist or not. Final cure of the eruption was always achieved within a month or two. The reaction to tuberculin was positive but of variable intensity. In a few cases there was a necrotizing reaction to tuberculin within 8–12 h, with bulla formation—possibly an Arthus-type reaction.

Two remarkable features were observed. The first was the simultaneous onset of papulonecrotic tuberculide and so-called idiopathic gangrene of the extremities in young adult Africans (Fig. 1). This occurred in seven of our cases (8%). The progress of both conditions was arrested by antituberculous therapy. The tuberculous origin of the arterial obstruction is difficult to confirm, but appears probable because of the association of the lesions and their concomitant response to antituberculous therapy (Findlay & Morrison, 1973).

The second feature, seen in four cases, was the development of lupus vulgaris from papulonecrotic tuberculides. These lesions consisted of all degrees of transition from papulonecrotic tuberculides to multiple annular self-limiting patches of lupus vulgaris and some larger plaques of progressive lupus vulgaris (Figs. 2 and 3). *M. tuberculosis var. hominis* was cultured from the lesions of two of these cases, and one patient had a super-infection with *M. kansasii*, an organism that flourishes on damaged tissue.

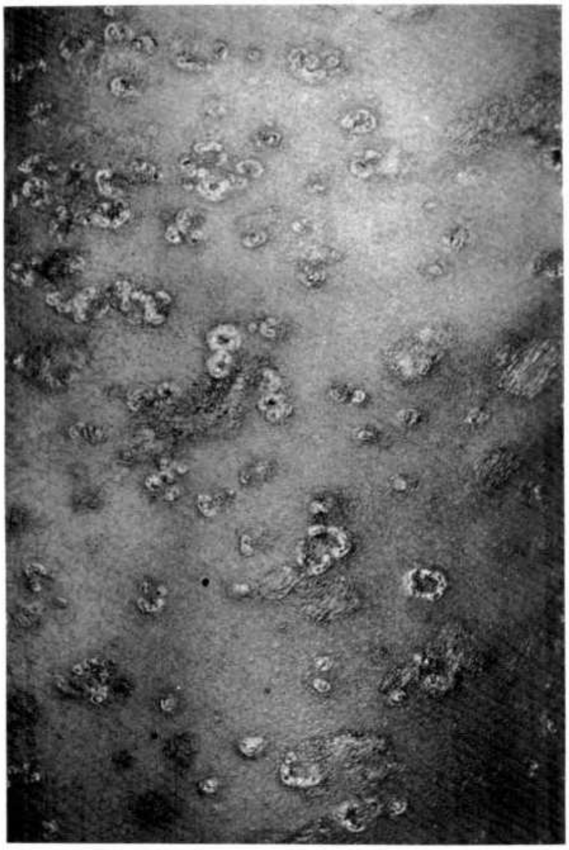

FIGURE 2. Papulonecrotic tuberculides on the thoracic wall with development of multiple annular patches of self-healing lupus vulgaris.

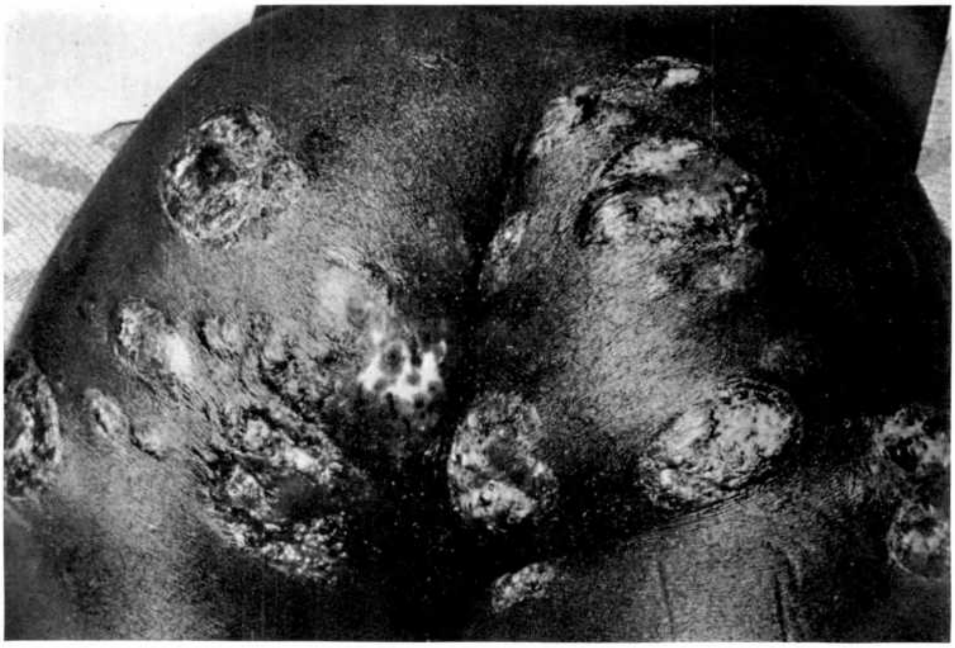

FIGURE 3. Buttocks of a patient with papulonecrotic tuberculides developing into multiple plaques of lupus vulgaris.

Histology

Eighty-three skin biopsies were available from sixty-nine cases of papulonecrotic tuberculide. In the early lesions an Arthus-type of reaction preponderated with swelling of the arteriolo-capillary endothelium in the dermis, intraluminal collections of polymorphs and fibrin, and some penetration and extravasation of cells through the vessel walls (Fig. 4a). There was vessel damage with a perivascular accumulation of neutrophil nuclear fragments, oedema and extravasated red blood cells (Fig. 4b). This was followed by a perivascular collection of mononuclear cells. In early stages these reactions appeared somewhat scattered, but in older lesions a well demarcated cone of necrotic tissue formed, extending from the dermis to the epidermis (Fig. 5a). This cone was gradually cast-off and epithelioid and giant cells gathered around its periphery (Fig. 5b). Exceptionally, some lesions failed to resolve at this stage, but developed into spreading plaques and annular granulomata. These showed the histological appearance of tuberculous follicles with central necrosis, surrounded by epithelioid cells, lymphocytes and giant cells (Fig. 5c). *M. tuberculosis* was cultured from these lesions in two of the four cases.

Changes in complement (Table 3)

Serum anti-complementary activity was determined in five cases with untreated papulonecrotic tuberculide by the method of Hook & Muschel (1964). In this procedure the heat-inactivated serum from the patients was added to guinea-pig serum, and the anti-complementary activity expressed as a decrease in the total haemolytic complement titre. The titre is expressed in 50% haemolytic units of complement per millilitre of serum (CH50/ml), representing the reciprocal of the amount of serum required to lyse 50% of $1\cdot5 \times 10^8$ optimally sensitized sheep erythrocytes.

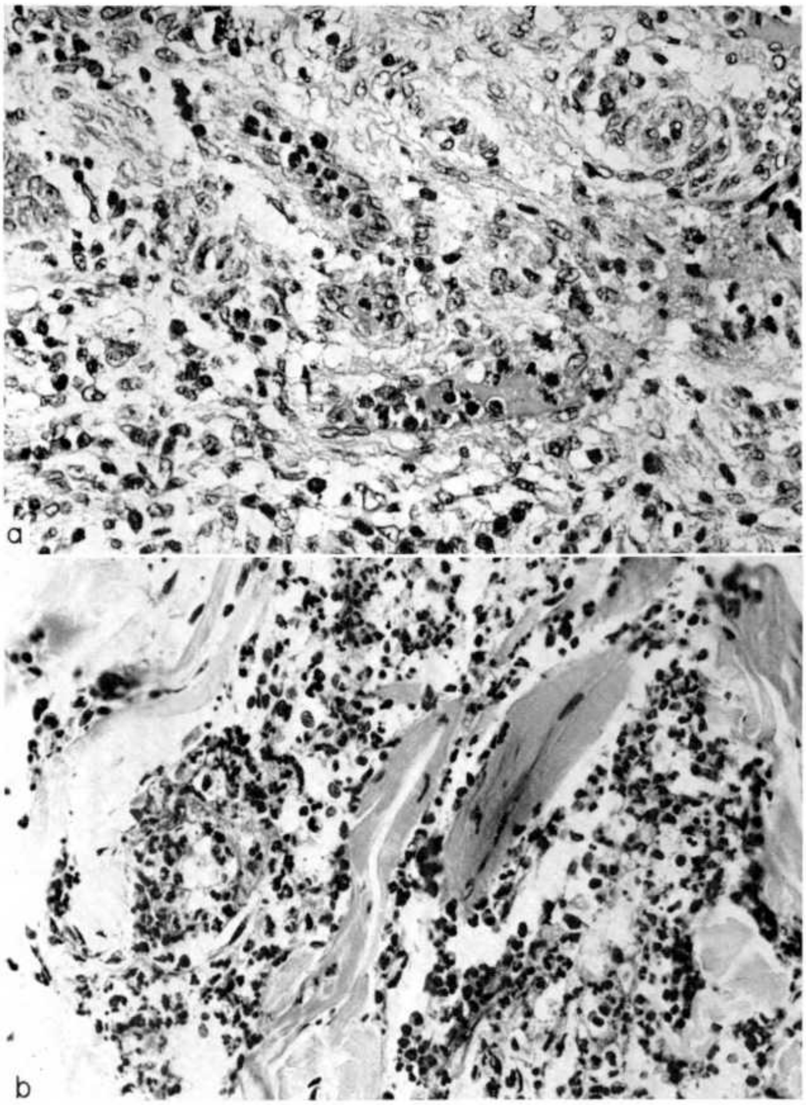

FIGURE 4. Early (Arthus) phase of papulonecrotic tuberculide. (a) Dermal vessels with intraluminal fibrin and neutrophils. (× 280). (b) Neutrophil response with damaged vessel walls (mid left and lower right), neutrophil nuclear debris and red blood cell extravasation. (× 280).

Complement activation was demonstrated by the *in vivo* conversion of C3 to C3c. The C3c concentration was determined semi-quantitatively by two-dimensional immuno-electrophoresis. Precipitation was effected with monospecific antiserum in agarose (Clarke & Freedman, 1967).

Anti-complementary activity and an increase in the C3c concentration signified the presence of immune complexes in all five patients tested.

The association of papulonecrotic tuberculides with lupus vulgaris and erythema induratum

During the past 17 years we have seen sixty-three patients with lupus vulgaris. The commonest form

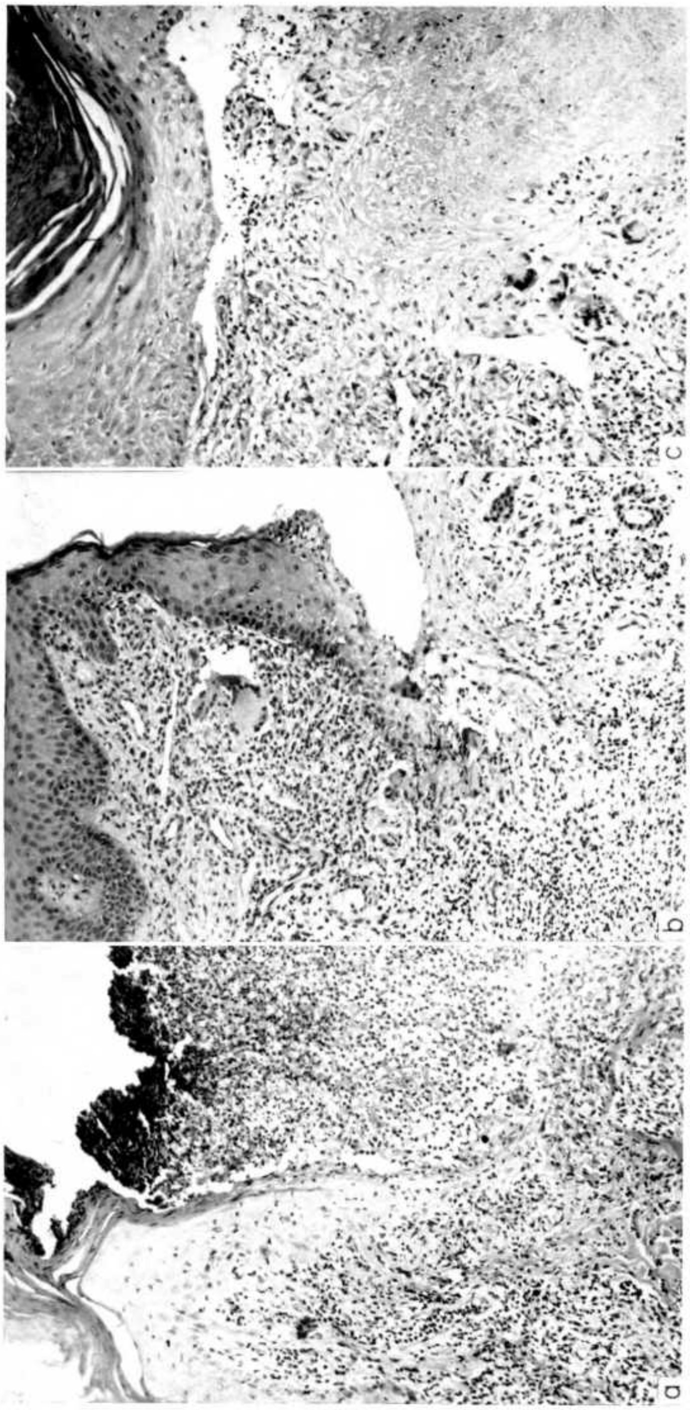

FIGURE 5. Transition from papulonecrotic tuberculide to lupus vulgaris. (a) A cone of necrotic cells with mononuclear cell infiltrate. (×96). (b) Epithelioid cells and giant cells around the crater of the cone (×96). (c) Tuberculous follicle with central surrounding necrosis epithelioid cells, giant cells and lymphocytes in a papulonecrotic tuberculide lesion that has developed into lupus vulgaris (×96).

TABLE 3. Complement activity in five cases of untreated papulonecrotic tuberculide

Case no.	Anti-complementary activity units		C3c concentration
	Initial guinea-pig CH50/ml	CH50/ml after addition of complement inactivated serum of patient	
1	420	360	+
2	420	40	+ + +
3	420	280	+
4	420	150	+ +
5	420	130	+ + +

(33%) started from the external nares. The limbs were next in order of frequency. A less common form followed scrofuloderma. Multiple plaques were rare, though some had evidently arisen from papulonecrotic tuberculides. An active tubercular focus was found in 11% of cases of lupus vulgaris (cf. 38% in papulonecrotic tuberculides).

Erythema induratum was seen in nine patients. Four of these also had papulonecrotic tuberculides. The pathogenesis of erythema induratum appeared to be similar to that of papulonecrotic tuberculide, but it occurred in larger vessels.

DISCUSSION

In human tuberculosis the serum usually contains antibodies against the antigens of the bacillus, even if they are only present in low titres (Boyden, 1958). From a tubercular focus, bacilli periodically enter the circulation. These usually die or are rapidly removed and killed by the reticulo-endothelial system. However, in patients with a high titre of precipitating antibodies against *M. tuberculosis*, these bacillary emboli are opsonified with antibody and complement, which alters their physical properties. Thereupon they settle out preferentially in slow flowing capillaries in the skin and perhaps in the vasa vasorum of the smaller peripheral arteries. Complement activation in the complexes evokes a polymorphonuclear leukocyte reaction through release of leukotaxines. The opsonified bacilli are phagocytosed by the neutrophils which remove the antibodies and complement, without reducing the viability of the bacilli. Subsequently, the polymorphs disintegrate and release proteolytic enzymes causing necrosis with destruction of the vessels. The consequent mononuclear cell response usually destroys the bacilli and the lesion heals with scarring. If the organisms survive they may lead to the development of lupus vulgaris. From such lesions *M. tuberculosis* can readily be cultivated. Subsequent monocyte activity may cut short lupus vulgaris at an early stage. We therefore suggest that the papulonecrotic tuberculide represents an Arthus reaction followed by a delayed hypersensitivity response, arising from the intravascular release of *M. tuberculosis*. A strong intradermal response to tuberculin is not an essential requirement for the forming of papulonecrotic tuberculides. When circulating antibodies are raised, skin lesions may result from an Arthus phenomenon, a delayed hypersensitivity reaction or a mixture of both (Chase & Macher, 1971; Ovary & Tigelaar, 1971). In some lesions histological evidence of fresh Arthus reactions may be seen among the established mononuclear infiltrate.

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Deoxyribonucleic acid (DNA) in cattle skin washings

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SUMMARY

Existing techniques have been modified to enable the estimation of DNA in skin washings from cattle. The technique evolved is described including the separation of a naturally occurring fluorescent substance in the skin washings.

The present work was undertaken to develop a technique for the estimation of small amounts of deoxyribonucleic acid (DNA) in cattle skin washings, in connection with studies of the mode of activity of the sebaceous glands which reputedly secrete in a holocrine manner (Ranvier, 1887).

MATERIALS AND METHODS

Collection of skin washings

Twenty-four hours before experimentation, an area approximately 40 × 10 cm on the dorsal surface of the back of twelve Ayrshire castrated bull calves aged from 6 to 9 months was shaved. At the beginning of each experiment this area was cleaned by washing three times with cotton wool soaked with 10% methanol, and skin washes were collected at given time intervals thereafter. The washings were collected using a tufnol cup with a rubber rim and an internal area of 28 cm^2. The cup was placed against the skin and 10 ml of solvent were added and gently agitated for 1 min with a glass rod. The solvent was removed with a Pasteur pipette and stored in glass bottles. Two such samples were combined for chemical analyses giving an amount per 56 cm^2 of skin.

The solvent used for sample collection was methanol in water, the optimum concentration of which was determined by estimating the recovery of DNA previously added to the skin surface. Immediately after initial cleaning of the skin of four calves, 5 μg were added to areas on each using a micro syringe. Six hours later skin washings were collected as described above, using 10, 20, 50 and 100% methanol. In this experiment, however, the samples were not pooled and the amounts per 28 cm^2 were determined. Suitable 'blank' skin washings were taken from each animal from areas to which DNA was not added. The samples were analysed for DNA content and the percentage recovery was determined.

Filtration

Each skin washing was passed through a membrane filter (Amicon Ltd) in order to remove salts and other low molecular weight substances. In the initial experiments an XM 100 A filter with a retention above 100,000 mol. wt was used. Subsequently, however, a UM 20 E with a retention of 10,000–20,000 mol. wt was selected for routine use. All samples were diluted with distilled water to approximately 10% methanol prior to filtration, to prevent damage to the membrane filters. The residue on the filter was washed with 10 ml of distilled water and made up to a final volume of 1 ml. This solution was transferred to a stoppered glass test tube and shaken for 30 min with 3 ml of chloroform to remove substances which fluoresce when exposed to UV light. 0·2 ml of this final solution was subsequently used for the analysis of its DNA content.

Chemical analysis

Estimation of DNA. The technique finally adopted was a modification of that of Kissane & Robins (1958) as adapted by Hinegardner (1971). The fluorescence produced when 3,5-diaminobenzoic acid (DABA) reacts with the deoxyribose exposed after removal of the purine bases by hot acid hydrolysis was measured fluorometrically. Of the concentrated skin washings 0·2 ml was transferred to a 10 ml conical tube and taken to dryness in a vacuum oven at 60°C. A distilled water blank and two standards of 1 and 2 μg DNA were treated in the same way. The aqueous standard was prepared from Sigma grade V DNA from Calf thymus (Sigma, London), with the addition of a few drops of ammonia to aid solution. This standard solution contained 1 mg DNA/ml as determined from the phosphorus content of the DNA (Sigma Chemical Co. assay). A 1:10 dilution of this solution (1 μg/10 μl) was used as a working standard. Aliquots ranging from 0 to 2 μg gave excellent linearity when subjected to the analytical technique. When the tubes were dry, 0·2 ml of a DABA (Ralph N. Emmanuel Ltd) solution (0·4 g/ml H_2O) was added to each tube which was then placed in an oven at 60°C for 45 min. The DABA used in the analysis was further purified as outlined by Hinegardner (1971) with the exception that an additional decolourization with charcoal was found to be necessary. The tubes were cooled to room temperature and 1·5 ml of N HCl were added to each. The contents, after thorough mixing, were transferred to quartz cuvettes and read in a fluorimeter (Locarte MK4) using an LF1 (254–400 nm) filter in the primary and an LF7 (510 nm) in the secondary position. The fluorimeter scale was set to read zero with the distilled water blank and to read 100 divisions with the DNA standard.

Initially a 2 μg standard was used, but later a 1 μg standard was adopted for greater sensitivity.

Accuracy of estimation of different amounts of DNA. In order to test the efficacy of the method to estimate varying amounts of DNA in cattle skin washings, the following procedure was used. The amount of DNA in cattle skin washings should vary with the time elapsed between washings, assuming that the amount of DNA is related to the amount of sweat and sebum secreted. Skin washings were, therefore, obtained from four Ayrshire calves, treated as previously described. These washings were taken immediately after cleaning, to give the DNA content at zero time and subsequently 6, 12 and 24 h after cleaning, with the resting time of 24 h between experiments. Duplicate pooled samples were taken from each animal at each time.

Fluorescent material. Skin washings from cattle exhibited a natural fluorescence in UV light and it was found that the substance or substances responsible could be removed by extraction with chloroform. In an effort to find out more about this material, skin washings were obtained from four areas on four calves in the manner described above, after 6 h exposure to 40°C dry bulb (DB), 10°C wet bulb (WB) and similar exposure to 20°C DB, 12°C WB. The concentrated skin washings were washed with 3 ml of chloroform as before, and the chloroform fractions for each animal were combined. The chloroform was reduced in volume to approximately 1 ml and was then spotted on TLC plates coated with Silica Gel G and developed in 50% methanol. 10 μg of DNA were added to one of the plates to determine its position on the plate relative to the autofluorescent material. After development the autofluorescent material was eluted from the Silica Gel with methanol, and its UV spectrum was examined. 0·5 ml of the substance in methanol was applied to an LH 20 Sephadex column 24 cm long and with a diameter of 1 cm. 2·5 ml fractions were collected and their absorption at 260 nm together with their degree of autofluorescence was measured.

Bacterial analyses. Horecker (1966) has reported the presence of deoxy sugars in bacterial cell walls. Since skin bacteria are not removed by the filtration process described, the amount of fluorescence produced by other deoxy sugars and by different concentrations of bacteria when subjected to the analytical technique was measured. Rumen bacteria are readily available at the concentrations required and form a convenient source of bacterial DNA. Different dilutions of rumen bacteria were therefore analysed for DNA content and the concentration of skin bacteria per 56 cm^2, 24 h after cleaning the skin, was determined.

RESULTS

Yields of 1·18 ± 0·19, 2·85 ± 0·21, 3·78 ± 0·21, and 1·85 ± 0·25 μg DNA respectively were obtained after filtration through an XM 100 A filter, with 10, 20, 50 and 100% methanol, from 5 μg of DNA added to the skin 6 h previously. The highest recovery rate (75·6%) was obtained with 50% methanol, which was selected as the solvent for subsequent experiments. Using this solvent strength and a UM 20 E filter, a recovery of almost 90% was achieved, after correcting for a 'blank' skin wash taken simultaneously. The UM 20 E filter was therefore subsequently used in the estimation of DNA in skin washings.

Two deoxy sugars, deoxy glucose and deoxy galactose, 5 μg of each, gave readings of 40 and 74 divisions respectively on the fluorimeter in comparison with 100 divisions of deoxy ribose when taken through the method described.

Bacterial counts on the concentrated skin washes of four animals kept under normal byre conditions and taken from 56 cm^2 of skin cleaned 24 h previously gave the following figures: $1·0 \times 10^7$/ml, $3·2 \times 10^7$/ml, $3·0 \times 10^7$/ml and $2·2 \times 10^7$/ml. Analyses as for DNA on three concentrations of rumen bacteria, $2·5 \times 10^7$/ml, $2·5 \times 10^8$/ml, and $2·5 \times 10^9$/ml gave scale deflections of 0, 2 and 24 divisions respectively when read against 2 μg of DNA set to 100 divisions on the fluorimeter. It would seem reasonable to assume from these findings that concentrations of skin bacteria would have little influence on the technique developed.

The mean amounts (with standard error) of DNA found in skin washings taken at 0, 6, 12 and 24 h intervals from 56 cm^2 areas on four animals were 0·11 ± 0·02, 0·57 ± 0·10, 0·99 ± 0·08, and 1·23 ± 0·06 μg respectively.

The chloroform extracted material when separated on TLC plates and examined under a UV lamp revealed a diffuse fluorescent area close to the solvent front; the added DNA remained at the origin. The fluorescent material after elution into methanol absorbed strongly at 260 nm in the spectrophotometer. However, unlike DNA, the absorbance increased below this wavelength. When fractionated on a Sephadex column the first nine fractions showed no absorbance at 260 nm. The next four had an average absorption of 0·130, while fraction 14 had a very high absorption (0·778). Fraction 15 had an absorption of 0·154. Autofluorescence was only found in fractions 14 and 15. DNA when passed through the same column separately was cleared by fraction 7. The fluorescent material was present in much larger quantities on animals in an environment of 40°C compared with one of 20°C air temperature.

DISCUSSION

The results indicate that 50% methanol is a satisfactory solvent for DNA, producing no observable skin damage. The recovery of DNA added to the skin surface was improved by introducing a membrane filter which retained material of lower molecular weight. It seems likely, therefore, that some degradation of DNA takes place on the skin surface with the production of oligonucleotides of varying molecular weight.

The relatively high fluoresence produced by deoxy glucose and deoxy galactose indicates that interference is possible when other deoxy sugars are present. A possible source of such sugars is bacterial cell walls (Horecker, 1966). The present work, however, shows that the numbers of bacteria present on the skin surface are unlikely to appreciably influence the estimation of DNA using the technique developed.

The presence of deoxy sugars as such in skin washes seems extremely unlikely; they would, of course, if present, pass through the membrane filter, and could not interfere in the analyses. DNA was certainly detected on the skin surface, the amount present increasing with time. A paper dealing with the relationship between sebaceous gland activity and changes in the concentration of DNA on the skin surface is in preparation.

The nature of the fluorescent material in skin secretions is unknown. It has a different absorption pattern in the UV region than DNA and appears to have a high molecular weight, although the possibility of its being a simpler molecule attached to a protein cannot be discounted. Proteins have been shown to be present in sweat (Jenkinson, Mabon & Manson, 1974), and the fact that the amount of fluorescent material is very much higher in actively sweating animals seems to bear out this possibility. Its role, however, remains a matter for conjecture.

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Monolayer culture of cells from psoriatic lesions

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SUMMARY

A method for culturing monolayers of cells from psoriatic lesions is described. Cultures have been maintained for 3 to 8 weeks. Apart from keratinocytes, melanocytes and non-pigmented dendritic cells, two types of cell were observed which are not commonly seen in normal skin, viz. a cell with a basket weave type of cytoplasm and a cell with a very long, up to 500 μm, cytoplasmic extension.

Epidermal cell suspensions of guinea-pig and human skin can be prepared by trypsinization and cultured as monolayers by the method originally described by Cruickshank, Cooper & Hooper (1960) and subsequently used with success by Cohen & Szabo (1968) and Fritsch & Diem (1972).

This method has been used in our laboratory to culture both human and animal skin, but attempts with psoriatic skin have hitherto been unsuccessful. We have attributed this to the failure to obtain clean separation of dermis from epidermis because of the depth of the rete ridges. A modification of the method has now resulted in the successful culture of psoriatic skin as a monolayer.

METHODS

Lignocaine 1% anaesthesia was used and both traditional elliptical biopsy material and biopsies cut in a superficial plane to minimize the amount of dermis were obtained. From the former it was necessary to remove fat and as much dermis as possible by means of fine curved scissors. The specimen size was approximately 10 × 6 mm in area. It was washed in sterile normal saline until free from blood and then placed in a small petri dish and minced to very small fragments by means of small curved scissors. The fragments were trypsinized for 1 h at 37°C in 10 ml of a solution of 0·2% trypsin (NBC) in Dulbecco phosphate-buffered saline sterilized by Seitz filtration. The trypsinization was carried out in a universal container (volume 25 ml) with occasional brisk agitation. After incubation, the supernatant trypsin solution containing released cells was separated from the tissue fragments with Pasteur pipettes and centrifuged in a round bottom test tube at 600 *g* for 3 min. The cell button thus produced was resuspended in 0·16 ml of medium consisting of 70% Eagle MEM-buffered with Hepes (Biocult Laboratories) and containing 1% of 200 mM glutamine with 30% autologous serum; streptomycin and penicillin were added to give a concentration of 50 units of each per ml.

The suspension of cells in medium was placed in a Cruickshank chamber (Sterilin), volume 0·16 ml, and incubated at 35°C in the inverted position for 2 days, when the medium was changed for the first time. Subsequent medium changes were carried out at 3-day intervals. The cells were examined daily by phase contrast microscopy.

When subculturing was necessary it was accomplished by replacing the medium with 0·2% trypsin and incubating for 15 min or until such time as gentle tapping had detached all the cells from the glass. The cells were recovered from the trypsin by centrifugation and were resuspended in sufficient medium, 0·32 ml, for two chambers only.

RESULTS

The change of culture medium at 2 days eliminated many non-viable cells. At this stage the cultures showed discrete cells attached to the glass and spreading, and there were occasional groups of three or four cells. By 6 days it was possible to identify many cells, including melanocytes and small colonies of keratinocytes; also present at this time were non-pigmented dendritic cells, similar to the type C cells of Cohen & Szabo (1968) (Figs. 1 and 2). By about 2 weeks, cells whose cytoplasm showed a basket weave appearance were present and were usually associated with small phase-dense dendritic cells (Fig. 3). In addition, there were cells with small nuclei, containing up to five or six nucleoli. Their cytoplasm showed single long processes up to 500 μm in length and of variable thickness with ruffled membranes at the tips (Fig. 4).

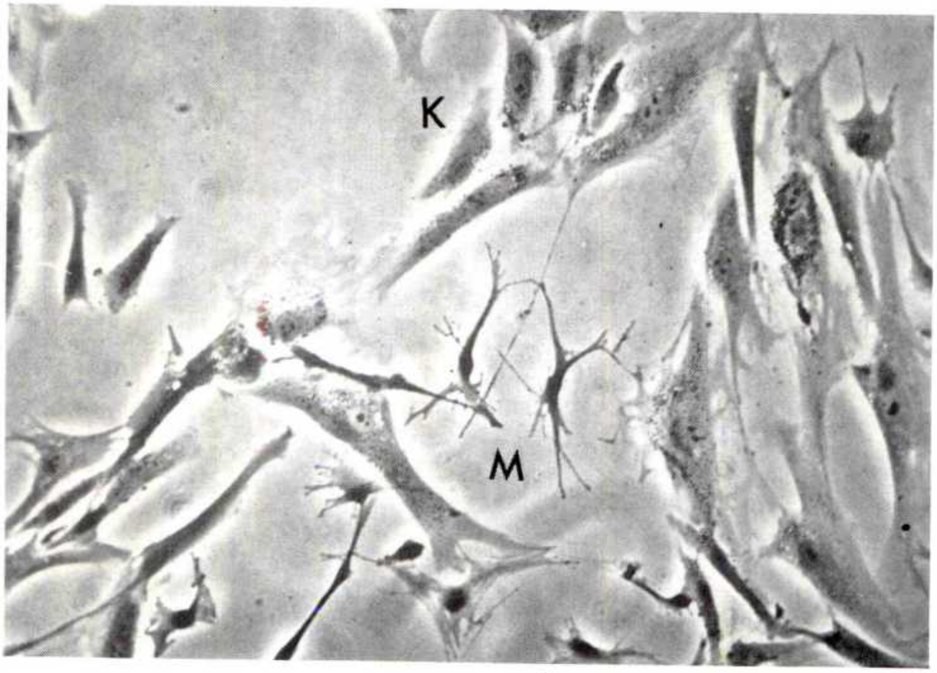

FIGURE 1. Keratinocytes (K) and melanocytes (M) at 15 days (× 200).

By 21 days the cultures had become confluent and required subculture (Fig. 5). In some cases cells with basket weave cytoplasm predominated. Fibroblast contamination occurred from conventional biopsy material, eventually outgrowing the cultures, whereas it was not prominent in cultures from superficial biopsies. Cultures without fibroblast contamination have been maintained for 3 to 8 weeks (Fig. 6).

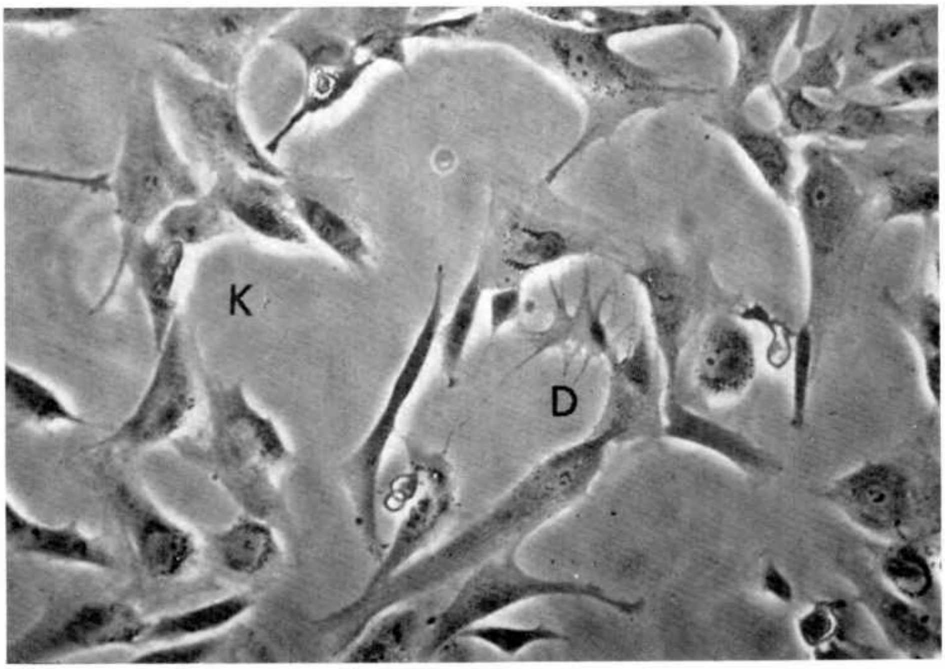

FIGURE 2. Keratinocyte (K) and non-pigmented cell (D) at 15 days (× 200).

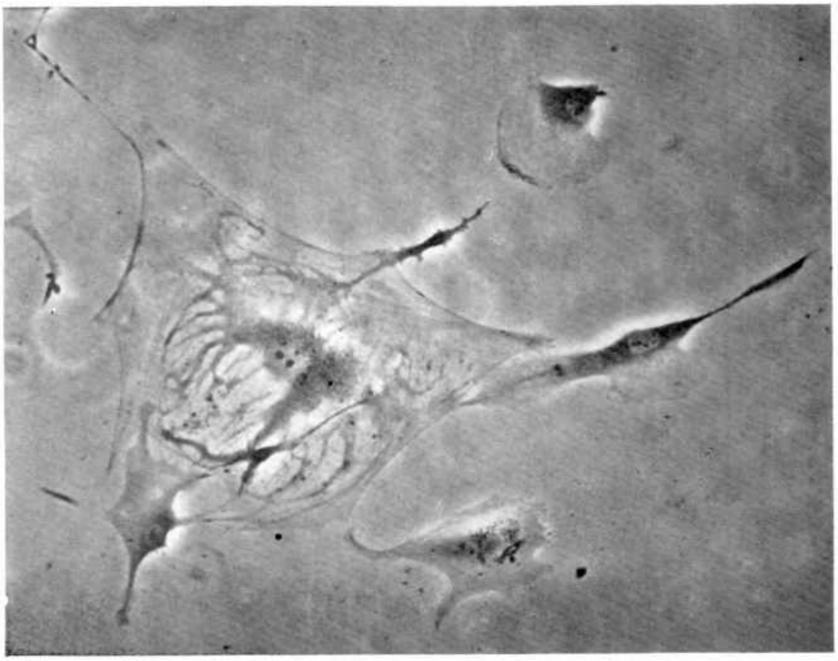

FIGURE 3. Cell with basket weave cytoplasm and associated phase-dense dendritic cells at 15 days (× 200).

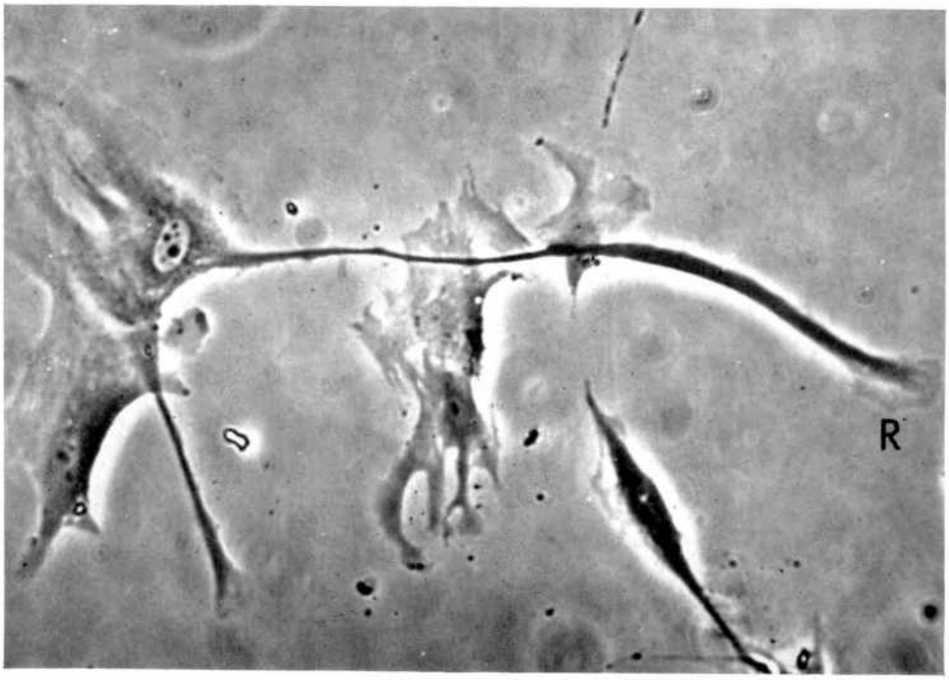

FIGURE 4. Cell showing long dendritic process with a ruffled membrane (R) at its tip at 15 days (× 200).

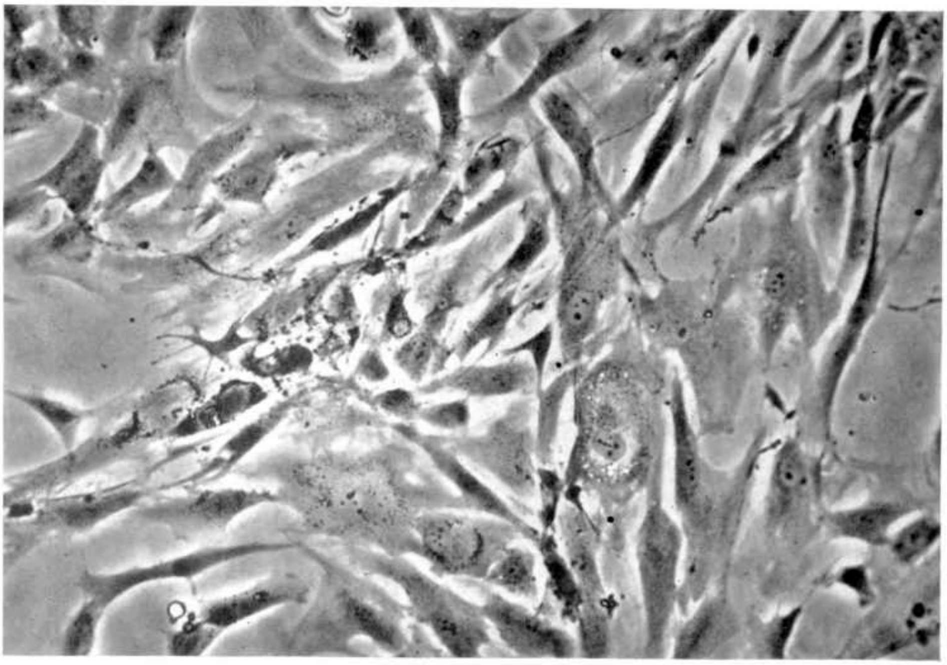

FIGURE 5. Confluent culture at 20 days (× 200).

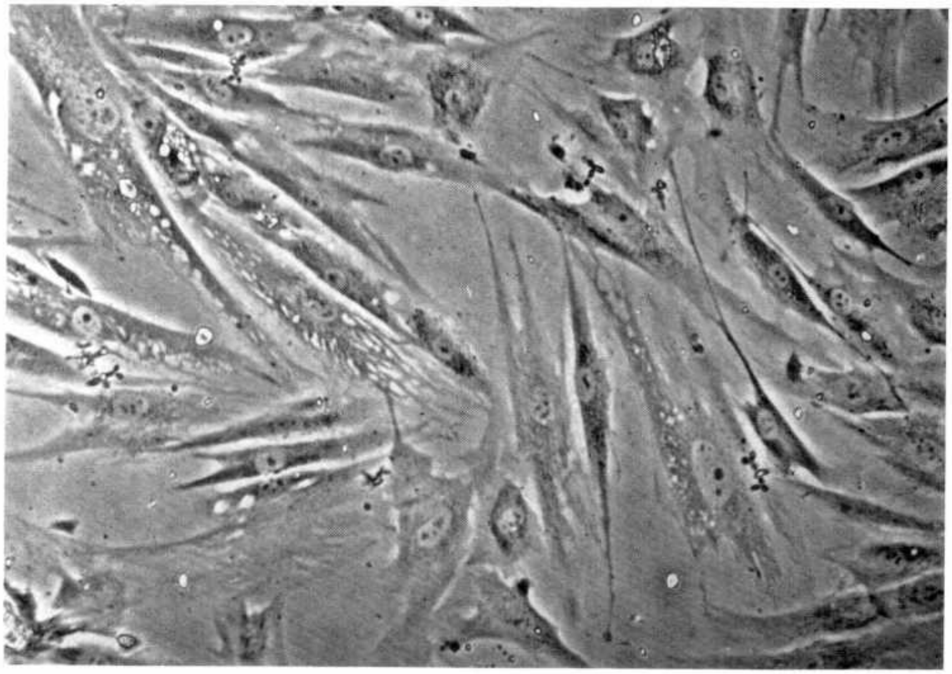

FIGURE 6. Confluent culture at 54 days (× 200).

DISCUSSION

Using this method we have been able to culture monolayers from biopsy specimens of eight patients with psoriasis. The success is probably due to the following technical modifications. Normal skin exposed to trypsin will split at the basement membrane level with the release of cells from this region, whilst psoriatic skin does not behave in this manner because of the depth of interdigitation of the rete ridges and dermal papillae. However, by reducing the specimen to small fragments for trypsinization a release of cells is obtained. Due to the small size of biopsy specimens and the limited number of viable cells obtained, the small volume of the Cruickshank chamber is a valuable contribution in establishing the optimum concentration of cells for initial survival. The use of a Hepes-buffered medium gives accurate control of pH in the early stages of establishing the trypsinized cells in culture.

The cells, present in profusion, with basket weave cytoplasm are probably keratinocytes and are occasionally seen in normal cultures. They may well represent an abnormality which is characteristic of imperfectly maturing keratinocytes in psoriasis and the possibility must be considered that their appearance is influenced by the presence of the small phase-dense dendritic cells. The latter, although occasionally obtained from normal skin, seemed to be present in greater profusion in psoriatic cultures. As judged by current studies they are probably not Langerhans' cells. The nature of the cells with single long dendritic processes remains to be established. They did not have the appearance of any cells usually seen in dispersed cell cultures of normal human or guinea-pig skin.

This method presents an opportunity to study cell behaviour in psoriasis. It is possible to compare and contrast cell cultures of psoriatic skin, apparently normal skin from psoriatic patients and healthy skin. Dispersed cell culture techniques also provide an opportunity to study histochemical characteristics (e.g. ATPase, DOPA, leucine aminopeptidase), cell interactions, cell division, chromosomal changes and the influence of potentially therapeutic substances.

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Immunoglobulin and complement deposits in the skin in inflammatory facial dermatoses—an immunofluorescence study

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SUMMARY

Biopsy material from ninety-one cases of inflammatory facial dermatoses was stained by the direct immunofluorescence method. The presence of immunoglobulins and complement in a distinct basement membrane band was seen only in lupus erythematosus. Negative results were found in thirty-five cases of rosacea.

The presence of immunoglobulin and complement bands at the dermo-epidermal junction in lupus erythematosus (LE) as shown by the direct immunofluorescence (IF) staining method was first described by Burnham, Neblett & Fine (1963). Many subsequent investigators have confirmed these findings, which have become the basis of a diagnostic procedure by which LE may be differentiated from other inflammatory dermatoses. Although IF staining in pemphigoid (Beutner, Chorzelski & Jordan, 1970; Jordan, Triftshauber & Schroeter, 1971) and cicatricial pemphigoid (Bean *et al.*, 1972) also show basement membrane localization of immunoglobulin and complement, their patterns are morphologically distinct from the LE band.

In 1969, Baart de la Faille & Baart de la Faille Kuyper described junctional fluorescent patterns of immunoglobulin and complement staining in twenty-one of thirty cases of rosacea. These were stated to be morphologically similar to those of LE and in ten cases the uninvolved skin showed the same findings. Comparable results were described by Salo (1970) and both authors considered that LE and rosacea might be related conditions.

Jablonska, Chorzelski & Maciejowska (1970) also described LE-like fluorescent bands in thirty-five of fifty-one cases of rosacea and in all (eight) cases of facial telangiectasia. They stressed the need for caution in the interpretation of IF results in facial lesions.

These reports cast considerable doubt upon the diagnostic value of the IF test in LE. We, therefore, describe the results of direct IF staining in ninety-one cases of facial dermatoses seen over a 2-year period.

MATERIALS AND METHOD

Elliptical facial biopsies from ninety-one patients were divided, half was fixed in 10% formol saline, processed and stained for light microscopy, and half rapidly frozen in liquid nitrogen, stored at −70°C, mounted in OTC medium and sectioned at 4 μm in a cryostat at −20°C.

Commercially obtained fluorescein isothyocyanate (FITC) conjugated antiserum to human immunoglobulins IgG, IgM and IgA (Wellcome reagents), complement (C_3), and fibrin (Hyland reagents), were used to stain all specimens. The cut sections were washed in phosphate buffered saline (PBS) pH 7·2 for 30 min and air dried before incubation with the various conjugates for 30 min. Further washing with mechanical agitation in PBS for 30 min (three changes) preceded mounting in buffered glycerol at pH 8. The sections were examined in a Leitz SM fluorescent microscope illuminated by a high pressure mercury vapour lamp (HBO 200), employing a BG38 red filter, an FITC-3 (Balzer) interference primary filter, and a K-510 secondary filter.

The FITC conjugated antisera had the following properties:

(1) Sheep antihuman IgG, P. 5·0 mg/ml, Ab.P. 0·39 mg/ml, F/P ratio 3·6 molar, diluted to 1:20.
(2) Sheep antihuman IgM, P. 6·4 mg/ml, F/P ratio 2·1 molar, diluted to 1:10.
(3) Sheep antihuman IgA, P. 5·9 mg/ml, F/P ratio 2·4 molar, diluted to 1:10.
(4) Goat antihuman C_3, P. 52·1 mg/ml, Ab.P. 1·2 mg/ml, F/P ratio 2·1 molar diluted to 1:30.
(5) Rabbit antihuman fibrin, P. 11·9 mg/ml, F/P ratio 2·2 molar, diluted to 1:30.

RESULTS (See Table 1)

In twenty-eight of the thirty-two cases of discoid lupus erythematosus, junctional bands of immunoglobulin were detected (Fig. 1), twenty-five demonstrated IgG, fifteen mixed immunoglobulins, and three IgM alone. Complement was present in twenty-two. Negative results were found in four cases, which by clinical and histological criteria were conclusively LE; one case had been inactive for several years. In three instances, with equivocal histological changes, the presence of a definite IF band assisted in early diagnosis.

TABLE 1. Immunofluorescence of facial dermatoses

	No. of cases	Positive IF band
Lupus erythematosus	32	28 (87·5%)
Telangiectasia	4	1
Rosacea	35	—
Eczema	12	—
Lymphocytic infiltration (Jessner)	5	—
Dermatomyositis	1	—
Granuloma faciale	1	—
Solar keratosis	1	—

The thirty-five cases of rosacea failed to show any significant immunoglobulin or complement deposits in the tissue. Apparent IF bands of weak intensity were present in the junctional zone of five tissues (Fig. 2), but on higher magnification (Fig. 3) this was resolved into a non-specific fibrillar band. This pattern was seen with one or more of the conjugated antisera, and in three cases of papulopustular rosacea it was confined to the junctional zone adjacent to the follicles.

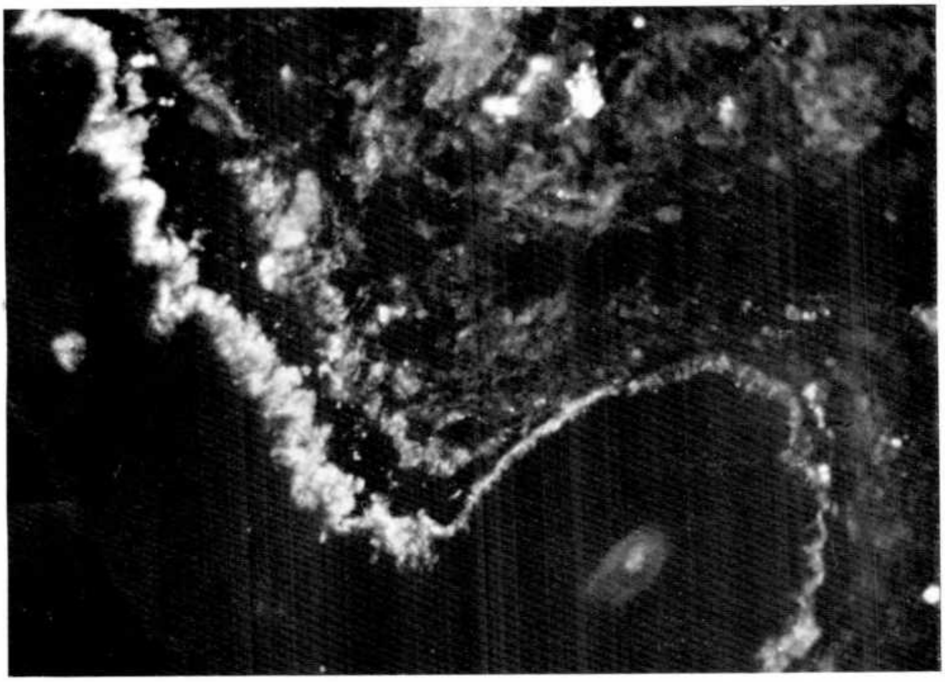

FIGURE 1. Discoid LE. Direct IF staining with antihuman IgG conjugated antiserum (×80).

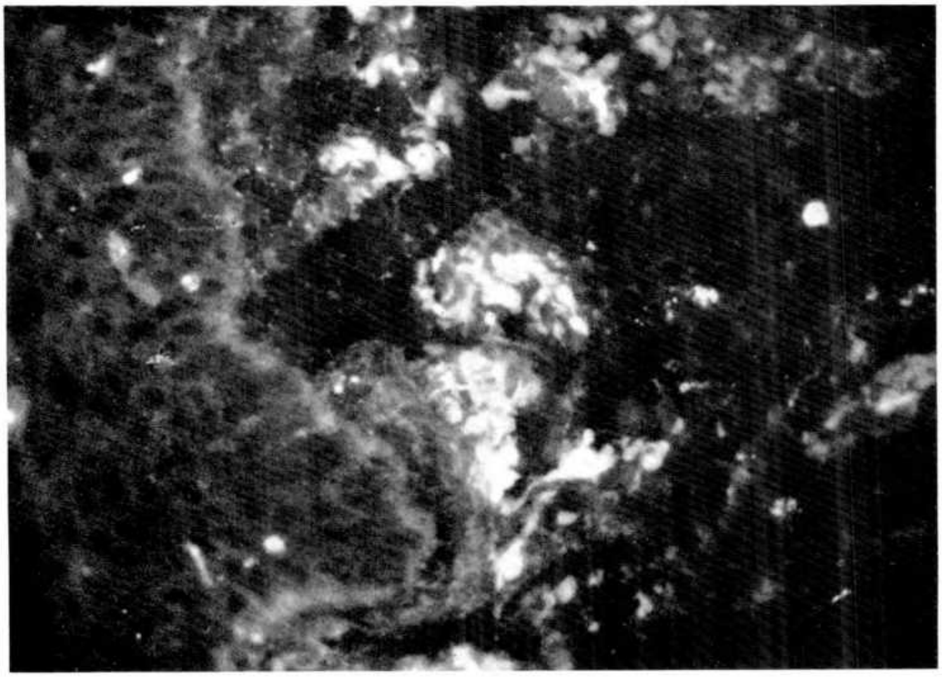

FIGURE 2. Rosacea. Direct IF staining with antihuman C_3 conjugated antiserum (×80).

Initially, in two patients considered clinically to have rosacea, positive LE fluorescent bands were demonstrated. Histologically, neither biopsy produced definite diagnostic features, but on follow-up both cases showed clinical and histological evolution into LE.

Four biopsies produced undiagnostic histology in which telangiectasia was the most prominent feature. The characteristic LE band (IgG, IgM, IgA and C_3) was found in one case.

The remaining twenty facial biopsies all produced negative results as detailed in Table 1. The group of twelve eczemas included four light-sensitive patients, two of whom had the clinical and histological features of actinic reticuloid (Ive *et al.*, 1969).

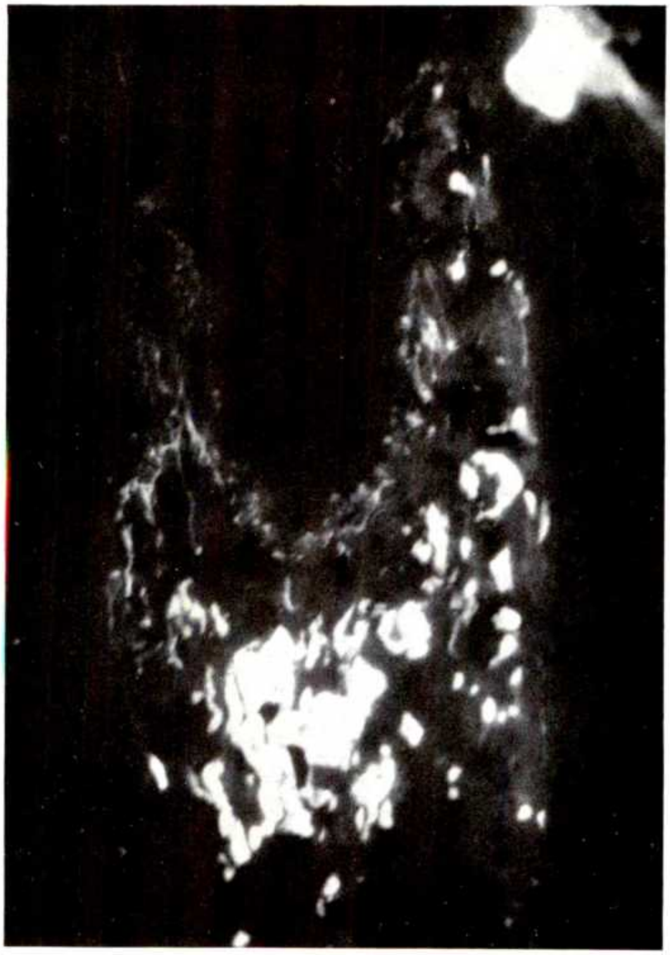

FIGURE 3. Rosacea. Direct IF staining with antihuman C_3 conjugated antiserum (× 320).

DISCUSSION

The histological changes of LE are more precise than those of rosacea and in assessing the latter group of patients the clinical and histological criteria for rosacea described by Marks & Harcourt-Webster (1969) in their review of 108 cases were employed. Histological diagnostic difficulties may still occasionally arise in both these conditions, and only if direct IF staining can be shown reliably to differentiate LE from other erythematous facial dermatoses will its diagnostic value be established in this particular situation.

In those clinically and histologically established cases of facial LE, nearly 90% demonstrated deposition of immunoglobulins and complement in the dermo-epidermal junction zone; whereas in the thirty-five cases of rosacea no such specific deposition was seen.

The apparently weak fluorescent bands seen in five cases of rosacea appeared identical to the fibrillar band described by Burnham & Fine (1971). Morphologically, we agree with Burnham that this appears to be formed by the compression of the dermal connective tissue against the junctional zone by inflammatory oedema and it seems particularly significant that this was found in the perifollicular area of three biopsies with marked papulo-pustular rosacea.

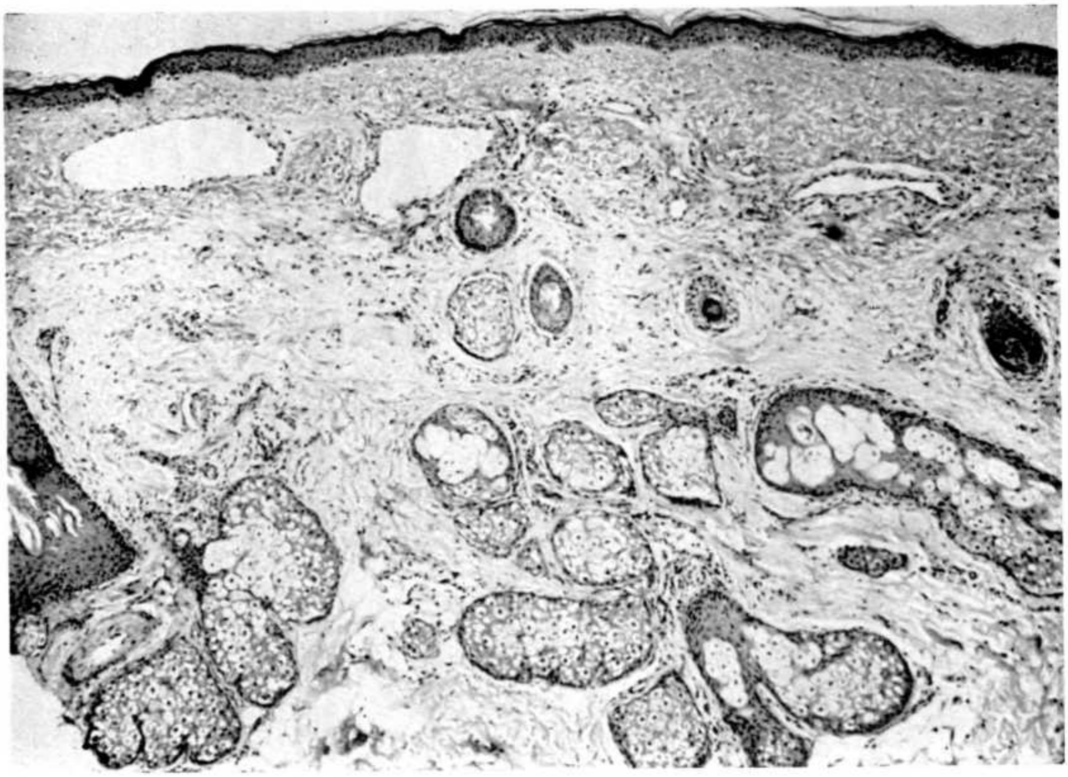

FIGURE 4. Undiagnostic facial telangiectasia (× 100).

The cases demonstrating telangiectasia did not show specific diagnostic features and were of the idiopathic or essential type. A positive band test was found in the biopsy of a negro woman of 45 years who presented with diffuse symmetrical telangiectatic erythema of the cheeks. Histologically (Fig. 4), features of LE were absent but telangiectatic rosacea was considered. However, rosacea in negroes is rare (Marks, unpublished observation) and, moreover, this eruption settled within 2 months with the use of a topical hydrocortisone preparation, after which the patient defaulted and a satisfactory diagnosis was not achieved.

We failed to find IF staining in a single case of juvenile dermatomyositis, although in occasional cases basement membrane zone IF bands have been reported (Chorzelski, Jablonska & Blaszczyk, 1969; Winkelmann, Jordan & de Moragas, 1972). In all other cases, including a large solitary actinic keratosis which resembled LE, IF staining also proved negative.

Our results, therefore, have shown a clear distinction between the results of direct IF staining in LE and rosacea, which are in complete contrast to the findings of Jablonska *et al.* (1970) and previous authors. In our experience, where positive LE immunofluorescent bands have been found in apparent cases of rosacea, subsequent follow-up has validated these initial IF results.

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Elastosis in chronic radiodermatitis

AN ULTRASTRUCTURAL STUDY

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SUMMARY

The electron microscopic study of six cases of chronic radiodermatitis from sun-exposed and unexposed skin, confirms the observation of elastosis previously described with light microscopy. The pathological findings are comparable with those found in actinic elastosis; increased width of the fibres, irregularity of their shape, increased density and granularity of the microfibrils and holes with many lobes in the matrix.

The findings may be due to transformation of elastic fibres into elastotic fibres or the formation of dystrophic elastotic fibres by dermal fibroblasts.

We have previously described the increase of dermal fibres in chronic radiodermatitis which occurred on sun-exposed and unexposed skin. These fibres were named 'elastotic fibres' for they possess many characteristics of elastic fibres staining with orcein and Weigert's resorcin–fuchsin, autofluorescence, lack of birefringence, digestion by elastase (Ledoux-Corbusier, 1967; Achten & Ledoux-Corbusier, 1968).

The present ultrastructural study was undertaken to compare these fibres with those described in actinic elastosis.

MATERIAL AND METHODS

Samples of skin were taken from six patients with typical chronic radiodermatitis.

The clinical characteristics of these cases are summarized in Table 1.

Each sample of skin was divided in two parts. The first, for light microscopical study, was fixed in Bouin's solution and stained with conventional methods; the second was cut into small blocks and fixed for 6h in 4% glutaraldehyde, in Millonig-glucose buffer at pH 7·4; the blocks were rinsed in the same buffer, followed by 1% osmium tetroxide in 0·1 M Millonig–glucose buffer (pH 7·4) for 1 h.

After stepwise dehydration in increasing concentrations of ethanol, specimens were passed through propylene oxide and embedded in epoxy resin (Epon). Semi-thin sections (approximately 750 nm thick) were immersed in NaOH–saturated ethanol for 1 1/4 h and rinsed with a series of alcohols of decreasing strength and finally with distilled water. They were stained with Taenzer–Unna acid

TABLE 1. Clinical characteristics

Case no.	Age and sex	Motive for radiotherapy	Localization of biopsy	Time since radiotherapy (years)	Characteristics of the irradiation		
					Nature	kV and filter	Dose (rad)
1. 3072/65 LA., L.	64 M	Basal cell carcinoma	Back	10	X-rays	25	5400
2. 3355/66 V.d.E., R.	66 M	Basal cell carcinoma	Back	12	X-rays	45 0·7 mmAl	5500
3. 72A 1372 DE., L.	74 F	Squamous cell carcinoma	Forearm (anterior surface)	20	X-rays	85 1 mm Al	4000
4. 94.428 DR., F.	79 M	Lupus vulgaris	Sub-mental	50	X-rays	Repeated irradiations during 5 years	
5. 5535/72 JE., G.	67 M	Lupus vulgaris	Cheek	56	X-rays Radium X-rays	?	
6. 2071/72 BO., M.	66 M	Sycosis of the beard	Sub-mental	40	Radium	?	

orcein (3 min) and metanil yellow 0·25% (15 s). Ultrathin sections (100 nm) were cut with an Ultramicrotome Reichert Om U2, stained with uranyl acetate (5%, 15 min), lead citrate (Reynolds, 10 min). The specimens were carbon-coated and studied with an electron microscope (Philips EM 300) operated at 60 kV.

RESULTS

The ultrastructural changes observed in the dermis of these six cases of chronic radiodermatitis are not uniform and are even variable for the same individual.

The samples examined at low magnification (×3300) show elastotic fibres occupying most of the dermis. They are often long and sinuous (Fig. 1), with a diameter varying from 2 to 6 μm. Sometimes they are packed together (Fig. 2). Their outline is regular in cases 1, 2 and 3, but irregular in cases 4, 5 and 6, with deep recesses filled with a fine threadlike and granular material (Fig. 3).

The background matrix of the elastotic fibre is usually translucent and homogeneous (cases 1, 2 and 3). However, in cases 4 and 5, it looks more dense and finely granular. Sometimes both appearances are present together. The elastotic fibres of case 6 have a very characteristic 'lace-like' pattern with round 'holes' or oblong multilobular clear lakes in the matrix, arranged in lines along the axis of the fibres (Fig. 4). These holes, surrounded by fine dense grains, contain one or more electron-dense round bodies (Fig. 5).

The elastotic microfibrils are more dense in cases 1, 2 and 3. In cases 4 and 5, they are also thickened and appear as masses of dense grains, so that the elastotic fibres, in longitudinal section, look like 'tiger stripes' (Fig. 6). The microfibrillar fringe, normally present at the extremities of normal elastic fibres, is never seen in chronic radiodermatitis.

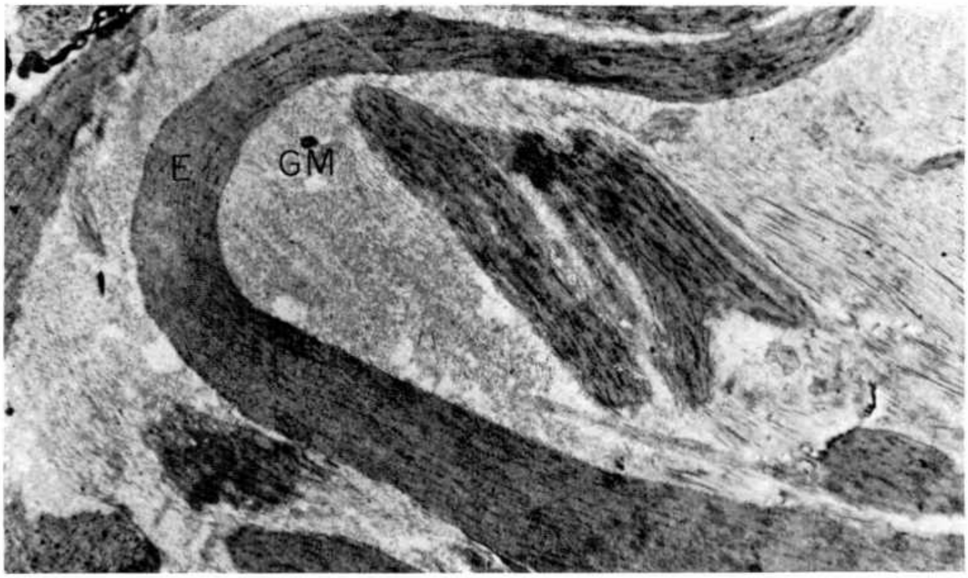

FIGURE 1. Long and sinuous elastotic fibre (E) surrounded by a fine granular material (GM). Absence of a microfibrillar fringe at the extremities of the cut fibres (× 5900—case 4).

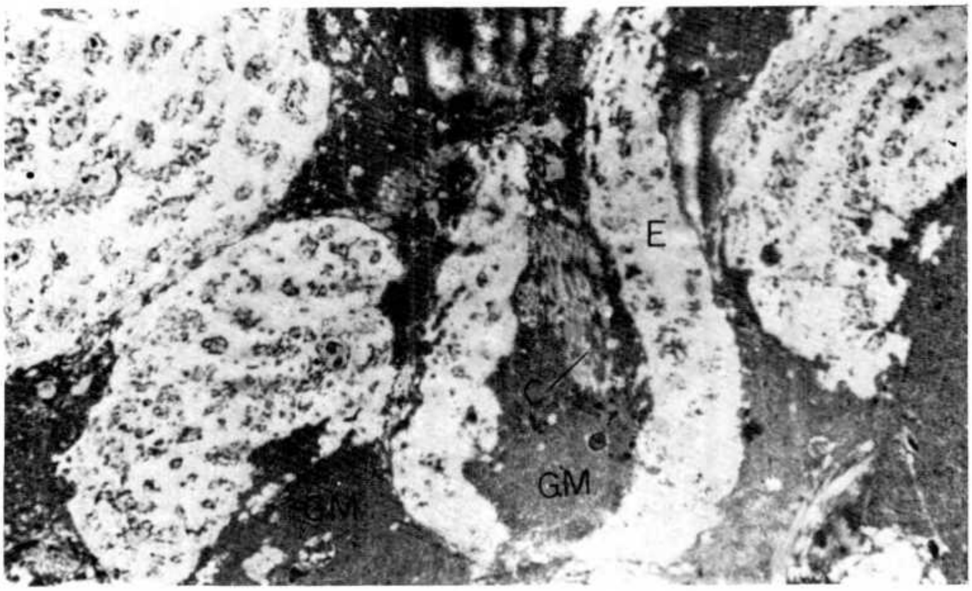

FIGURE 2. Broad elastotic fibres (E) packed together. Rare collagen fibres (C). Granular material (GM) very abundant in the extrafibrous spaces (× 3600—case 6).

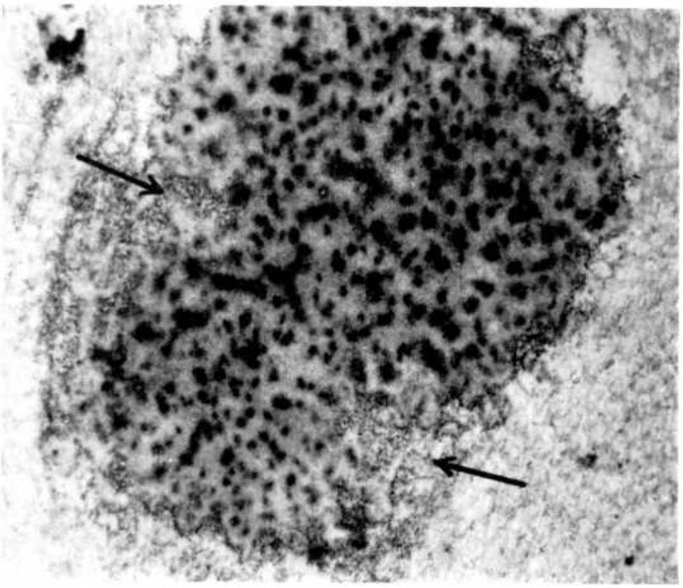

FIGURE 3. Elastotic fibre, transversely cut, showing thickened microfibrils. A fine granular material coats the fibre and penetrates deep recesses (arrowed) (× 24,000—case 4).

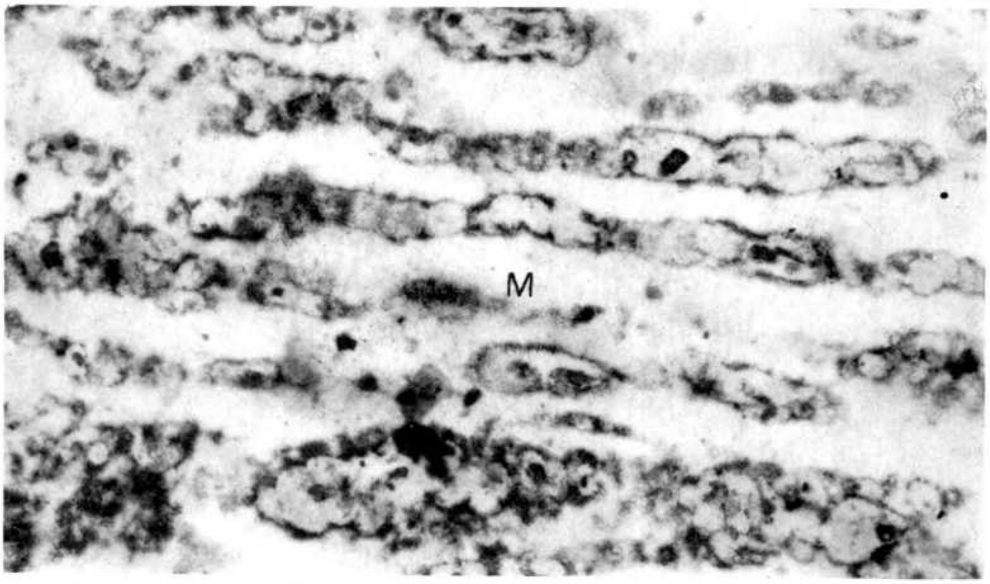

FIGURE 4. Lace-like pattern of an elastotic fibre with oblong, multilobular lakes in an electrolucent matrix (M). These, with a dark rim, are arranged in lines along the axis of the fibre (× 20,500—case 6).

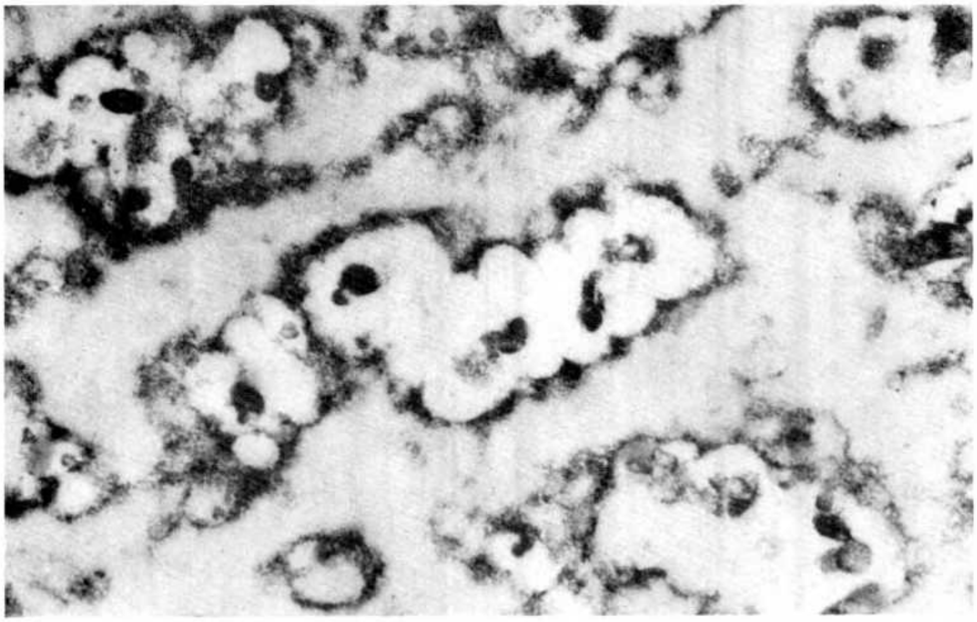

FIGURE 5. Multilobular holes surrounded by fine dense grains and containing one or more electron-dense round bodies (× 35,200—case 6).

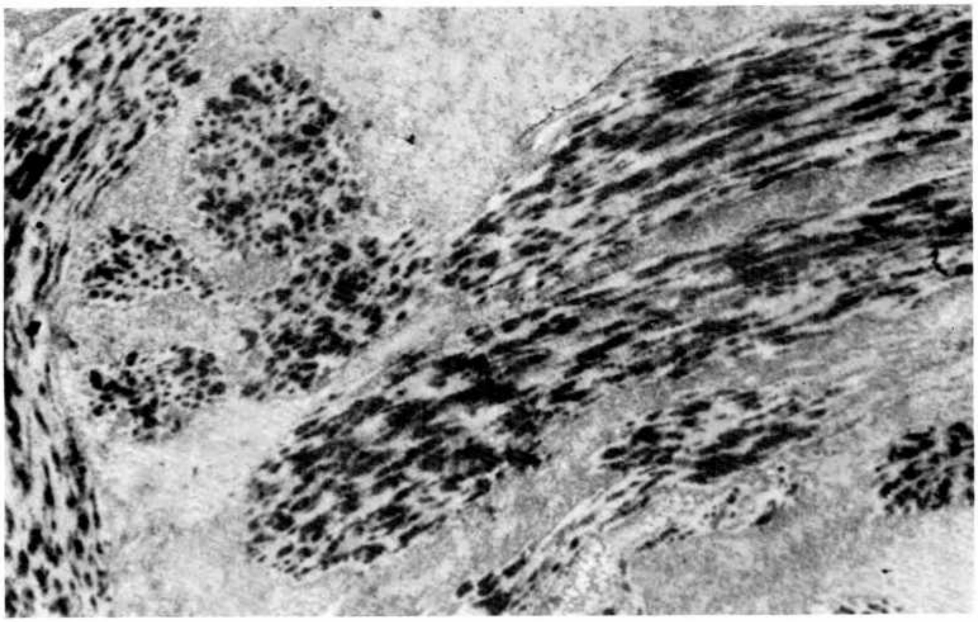

FIGURE 6. The elastic microfibrils are so dense that the elastotic fibres look like 'tiger stripes' in longitudinal sections. A fine thread-like and granular material is present in the extrafibrous spaces (× 8600—case 5).

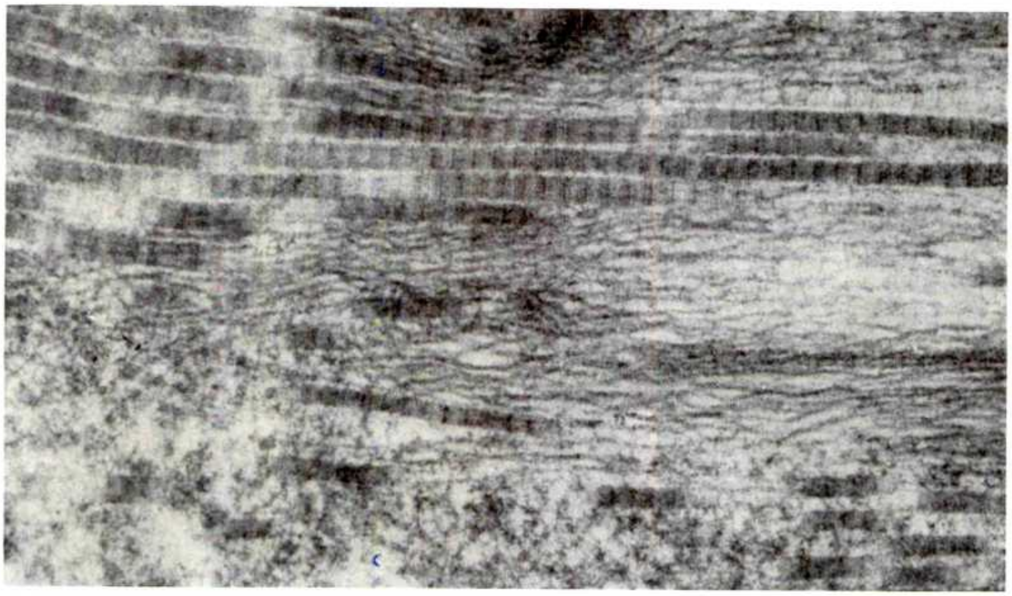

FIGURE 7. Locally, masses of microfibrils take the place of collagen fibres (×49,000—case 4).

A fine thread-like and granular material is always found, coating the elastotic fibres and penetrating the fibres in deep infoldings (Fig. 3). This material is also observed, at the surface of the fibroblasts and in the extra-fibrous spaces, and in case 6 the collagen fibre bundles are dispersed by it.

The collagen bundles are composed of a smaller amount of fibres than normally. The fibres' diameter may vary but are mostly normal (±100 nm) and the axial periodicity is maintained. In transverse sections, the centre of the fibres sometimes shows decreased staining. Locally, masses of microfibrils take the place of collagen fibres (Fig. 7).

The fibroblasts are decreased in number. Their ergastoplasm is, however, well-developed and microfibrils are often present in their cytoplasmic processes. Elastotic fibres are frequently observed in close vicinity to fibroblasts.

Amorphous droplets and irregularly shaped lamellar structures, isolated or grouped, are observed in cases 4 and 5. Their central core, amorphous and electron-transparent, is limited by the same finely thread-like and granular material already described. This material is stained with uranyl acetate and lead citrate. The width of these lamellar structures is approximately 180 nm (Fig. 8).

DISCUSSION

In chronic radiodermatitis normal elastic fibres are replaced entirely by elastotic fibres. They show morphological changes identical with those found in senile-actinic elastosis by Banfield & Brindley (1963), Braun-Falco (1964, 1965, 1968, 1969a, b), Berger & Walter (1967), Hashimoto (1967), Pierard, Kint & de Bersaques (1968), Danielsen & Kobayasi (1972). As in actinic elastosis, the changes are not uniform but vary with patients and with samples examined.

The abnormalities noted in the elastotic microfibrils and matrix seem to show a gradual evolution. In chronic radiodermatitis, the microfibrillar fringes disappear from the fibre's extremities.

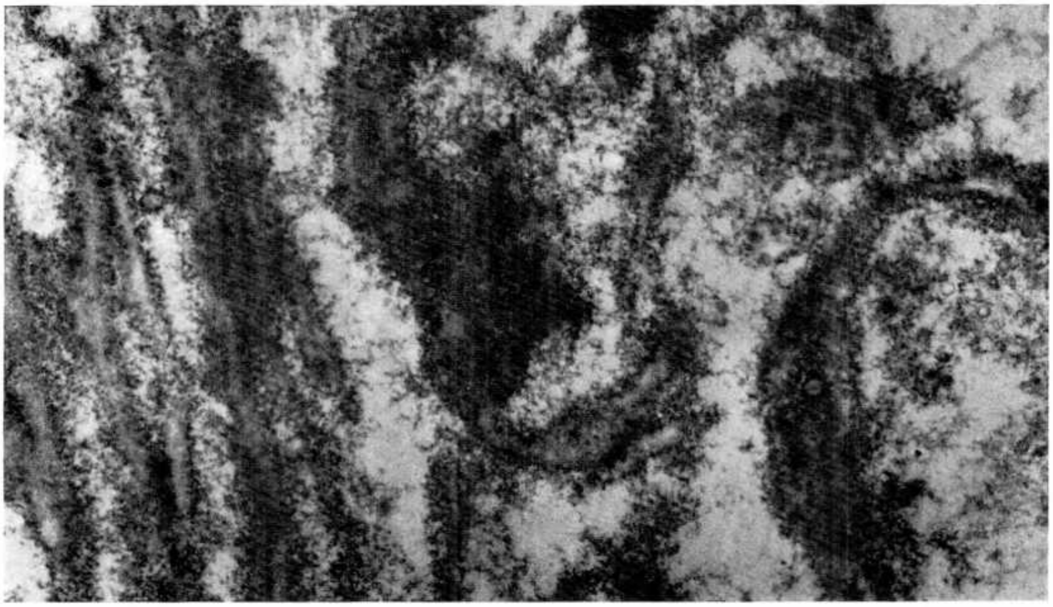

FIGURE 8. Lamellar structures, irregularly shaped, are observed. Their amorphous core is limited by a fine granular material (× 30,000—case 4).

The elastotic matrix is sometimes electrolucent as in a normal elastic fibre but sometimes light-grey and finely granular. Both appearances may be observed side by side. There is a variation in the staining of the matrix with lead and uranyl acetate which appears to depend on the size and maturity of the matrix (Jones & Barson, 1971). The lace-like pattern of the matrix, observed in case 6, has been well described by Pierard *et al.* (1968) in actinic elastosis. The oblong 'holes' with electron-dense bodies are similar to those observed by Danielsen & Kobayasi (1972). Their origin and nature are unknown. Their granular outline could not represent normal microfibrils cut transversely, for in longitudinal sections of the elastotic fibres, the granular appearance remains.

The fine thread-like and granular material found in chronic radiodermatitis has been reported in actinic elastosis where it coats the surface of the broad elastotic fibres, the collagen fibres and the fibroblasts (Mitchell, 1967). Mitchell considered it as degraded collagen and ground substance. Braun-Falco (1968, 1969a, b) felt that these granules could be the origin of the elastotic fibres and are comparable to the homogenous material observed during elastogenesis in tissue culture. Kobayasi & Asboe-Hansen (1972) stained a similar material coating the elastic fibres in scleroderma, using a periodic acid–silver proteinate technique which indicates a high content of glucosaminoglycans. These granules may be the 'elastic granules' described by Kadar, Gardner & Bush (1971) in elastogenesis.

The collagen fibres are decreased in amount as in actinic elastosis, but they show only minor variations in their diameter and staining reaction. In some places, they split into microfibrils. These changes, described in actinic elastosis by Niebauer & Stockinger (1965), Mitchell (1967) and Braun-Falco (1969b), probably represent collagen degradation, though Danielsen & Kobayasi (1972) considered them to be artefacts.

The fibroblasts are not numerous, but they have normal mitochondria, a well-developed granular endoplasmic reticulum and intra-cytoplasmic filaments. In actinic elastosis, Braun-Falco (1968), Kretzberg & Klingmüller (1968) and Ebner (1969) made similar observations and thought that these structures indicated active fibrogenesis. Braun-Falco (1968, 1969a, b) also noted the close vicinity between fibroblasts and elastotic fibres. The same proximity is observed in chronic radiodermatitis.

Two hypotheses, already proposed to interpret the development of actinic elastosis, could also suggest the morphogenesis of radiodermatitic elastosis.

The first is the transformation either of the collagen fibres (Tunbridge *et al.*, 1952; Teller, Vester & Pohl, 1957; Niebauer & Stockinger, 1965) or of the elastic fibres (Banfield & Brindley, 1963; Pierard *et al.*, 1968) or of both of them with incorporation of a granular material (Mitchell, 1967).

The second is fibroblastic activation by ultravoilet rays resulting in the formation of abnormal fibres which undergo degenerative changes (Braun-Falco, 1968, 1969a, b; Kretzberg & Klingmüller, 1968; Ebner, 1969).

In chronic radiodermatitis, there is nothing to suggest that collagen fibres are transformed into elastotic fibres. Moreover, the collagen and the elastotic fibres have quite different structures.

If the first hypothesis is right, the elastotic fibres would derive from the elastic fibres with which they have morphologically much more in common. However, in radiodermatitis, we neither found normal elastic fibres nor observed the transformation of elastic fibres into elastotic fibres.

On the other hand, some arguments make us believe that in chronic radiodermatitis the elastotic fibres could be formed by an active process; the granular material described is comparable with the elastogenic product found in tissue cultures, the fibroblasts present morphological signs of cellular activity and the lamellar structures observed look like abnormal 'elastic units' (Haust & More, 1966; Jones & Barson, 1971; Robert, Robert & Robert 1970; Robert *et al.*, 1971). The abnormal fibres would further undergo degenerative changes as in actinic elastosis. These changes depend on individual sensitivity, irradiation characteristics and also the time interval following the high-energy irradiation.

Further experiments, such as enzymatic digestion, would be necessary to establish the precise nature of the granular structures observed.

ACKNOWLEDGMENTS

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Black grain mycetoma

A STUDY OF THE CHEMISTRY, FORMATION AND SIGNIFICANCE OF THE TISSUE GRAIN IN *MADURELLA MYCETOMI* INFECTION

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SUMMARY

In a study of the black tissue grains formed in mycetoma caused by *Madurella mycetomi*, the evidence pointed to a tanned protein or melanoprotein as the main durable constituent of the grain cement. It appeared likely that the melanizing agent arose from the fungus, while the protein component came from the host. Resistance to treatment was considered to depend on the impenetrability of the cement so produced.

At present the only treatment available for advanced cases of black grain mycetoma is amputation of the affected part. As a rule this means the loss of a foot or leg, to the ruin of a labourer's working capacity. Prolonged chemotherapy has seemed to us to produce scarcely more benefit than bed-rest would do. We were therefore interested to discover any reason for the intractability of black grain mycetoma as compared with the actinomycotic mycetomas, where the therapeutic results have been much more satisfactory.

Even if the organism were killed by potentially valuable antifungal agents *in vitro*, it seemed that the grain which is formed closely about the fungus in the tissues could be acting as a barrier to fungicidal drugs. The grain itself thus seemed worthy of a preliminary study.

The grain is innocently stated to consist of a black cement. Its dark colour is sometimes described as being due to a melanin secreted by the fungus, though we could trace no evidence for this assertion. As to the cement itself, nothing appears to be known about its origin or properties, apart from an extreme hardness when dry. Our present report considers the fungal pigment, the cement, the intact grain and some possible methods of grain formation.

MATERIAL

Surgical specimens of three cases of mycetoma, yielding *Madurella mycetomi* on culture, were available for various parts of the study.

OBSERVATIONS

Tissue inhibition of fungal growth

In sections of the granuloma, the mycelium of *Madurella mycetomi* scarcely ever projected beyond the outer limits of the grain. However, on transferring a fresh mass of inflammatory tissue containing grains to normal saline solution, we found that the mycelium grew freely out of the black grains. This suggested that the fungus survived in the tissue under considerable adversity. On the outer surface of many tissue grains, histological sections showed a thin homogeneous mantle staining strongly with eosin. The eosinophil layer was selectively removed by tryptic digestion, while the brown-coloured cement of the fungus grain resisted trypsin.

By analogy with other types of eosinophilic halo around fungus cells in the dermis, we thought this could represent the layer of an antigen–antibody precipitin reaction. We concluded that the fungus required the protection of the cement to ensure its survival in the host tissue.

The fungal pigment in culture

Production of a diffusible brown pigment in Sabouraud's agar was well marked in the fresh isolates, but declined gradually in subculture. Addition of tyrosine and dihydroxyphenylalanine (DOPA) to the medium was without influence on pigment production. Catechol, however, led to a marked increase in pigmentation. When grown on water agar the fungal pigment was lacking, but commenced when a few catechol grains were placed near the growing fungus.

The pigment was readily leached out of the Sabouraud's medium by water. Thereafter, it was not taken up by chloroform, ether, acetone and several other fat solvents, but passed readily into alcoholic solutions. The iron alum test at pH 7 for phenols was negative on the pigment extract, only a brown gel being precipitated.

In slide cultures the pigment appeared as a diffuse colour in the moisture around the hyphae. Its origin could not be determined. It was not particulate.

From the absorption spectra of the alcoholic extracts, pigment appeared to be variably conjugated with the neopeptone of the culture medium. One fresh extract gave a curve suggesting a melanin-type polymer. It was clear from the spectra of several extracts that the composition was changeable, and showed a variable broad peak at 280 nm (Fig. 1). Separation of the pigment and other components on thin layer chromatograms gave rise to equivocal results which await later study.

We concluded provisionally that the pigment was a highly condensed phenolic tannin or 'melanin' which did not apparently correspond to any of the better known plant tannins of the flowering plants as judged chromatographically.

The fungal pigment in the tissue grain

While it was easy to extract pigment from the culture media, the tissue grains yielded relatively little of the total pigment present in the grain on aqueous extraction. The absorption curves of this brown extract resembled those of hardened protein (Hackman, 1953), not unlike that which we had already obtained from the extracts of the pigmented peptone-containing culture medium. This coloured solution from the grains showed a spectral shift on reduction by sodium dithionite (Fig. 2).

The fixed pigment component in the grain was seen on sections as an even yellow–brown staining of the grain cement. Histochemically, it behaved like melanin, being bleached by strong H_2O_2, and taking the silver staining reactions of melanin.

A few tests on bulk bleaching and digestion were undertaken on whole grains. 30% H_2O_2 bleached the grains slowly (36 h) while chlorine-type bleaches (diaphanol, Milton) did so more rapidly (2–12 h). The pigment was not liberated or removed by digestion with trypsin, pronase, hyaluronidase or

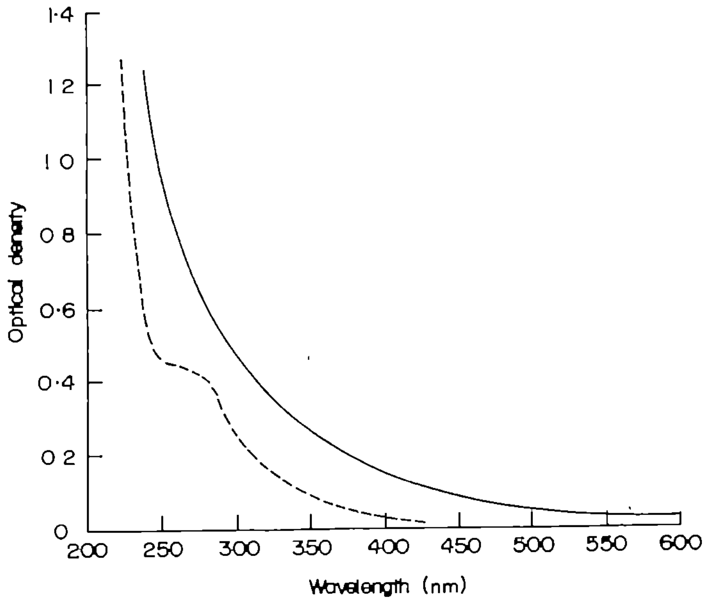

FIGURE 1. Absorption spectra of two somewhat different pale brown alcoholic solutions of fungal pigment extracted from culture medium of *Madurella mycetomi*. Solid line shows a melanin type curve. Broken line shows a plateau roughly around 260 nm, suggesting a conjugate with the peptone in the medium.

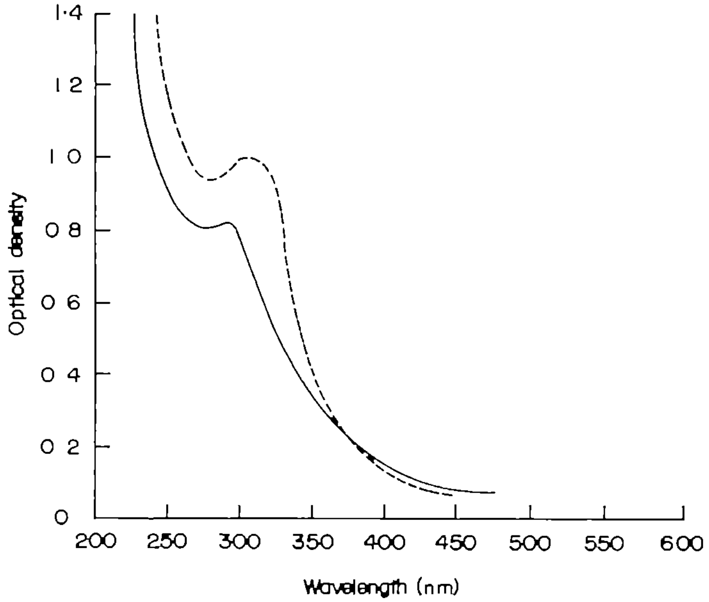

FIGURE 2. Fungal pigment absorption spectrum. Solid line, untreated; broken line, reduced with solid sodium dithionite.

chitinase, nor by 6 N HCl at 100°C in a sealed tube for 12 h. With 50% KOH for 2 h in a sealed tube at 160°C the grains were whitened. Where whitening occurred by whatever means, the grain became softer.

These tests supported the idea of a melanoprotein pigment, present in the grain largely in a fixed insoluble form as the so-called cement. Its origin from some function of the fungus seemed probable.

Staining properties of the fungus in the grain

The hyphal walls of the fungus embedded in the grain cement are readily stained with the PAS procedure. Prior bleaching of the surrounding cement itself with diaphanol overnight greatly enhanced the visibility of this reaction. A finding which we did not investigate further was a selective demonstration of the outline of the fungal cell walls by the reticulin procedure (ammoniacal silver nitrate) following H_2O_2 bleaching.

Structural polysaccharides in the grain

Because of its extreme resistance to digestion, the grain was investigated for its possible content of structural polysaccharides, since fungi are known to produce chitins in adverse circumstances.

Of the established tests for chitin, the Schulze reaction (zinc chloride–iodine) was negative; the whole grain and the grain on section were resistant to chitinase; crystals of chitosan sulphate could not be obtained; after drastic acid hydrolysis by several means and suitable preparation for gas–liquid chromatography, no evidence for chitin or other major polysaccharide was obtained. Controls with insect cuticle yielded abundant *n*-acetyl glucosamine as the expected breakdown product of chitin.

The histochemical periodic acid–Schiff reaction was consistently negative in sections of the grain. Destombes (1972) found otherwise, and stated that the proteinaceous cement stained homogeneously with PAS. At present this discrepancy remains unresolved.

Protein components in the grain

A suggestion was made by Champeau (1954) that the grain structure was filamentous, and that these filaments were observed to run in continuity with the granuloma round about. With Laidlaw's silver procedure for reticulin he claimed with some justification that the grain and the surrounding reticulin filaments were in continuity, and that the reticulin plunged into the melanin blocks. To avoid the hazard of false silver stains from melanin while attempting to show reticulin, we bleached the melanin but found thereafter that the 'reticulin' failed to stain.

Although Champeau (1954) did not state how this staining problem was overcome, his idea was not to be dismissed on these grounds alone (see below, electron microscopy).

Exposure to pronase for several hours caused the grains to cleave open to some extent but they were not digested. For protein extraction a few clean grains freshly removed from a patient were placed in a small volume of 0·1 molar phosphate buffer pH 7 with 1% each of sodium dodecylsulphate (SDS) and mercaptoethanol. Electrophoresis (SDS–acrylamide) yielded a small quantity of low molecular weight protein (15–20 thousand).

With cryostat sections of fresh grains, there was a diffuse attachment of IgG over the grain as judged by fluorescence microscopy. IgM and IgA were negative. There was no attachment of fluorescent immunoglobulin on sections of the fixed grain.

Electron microscopy

With the scanning electron microscope, the grain was shown to have a polypoid structure with a somewhat laminated surface (Fig. 3). The same picture was given even after the grain had been treated in 50% KOH at 160°C for several hours. The specimen remained stable in the highest magnifications

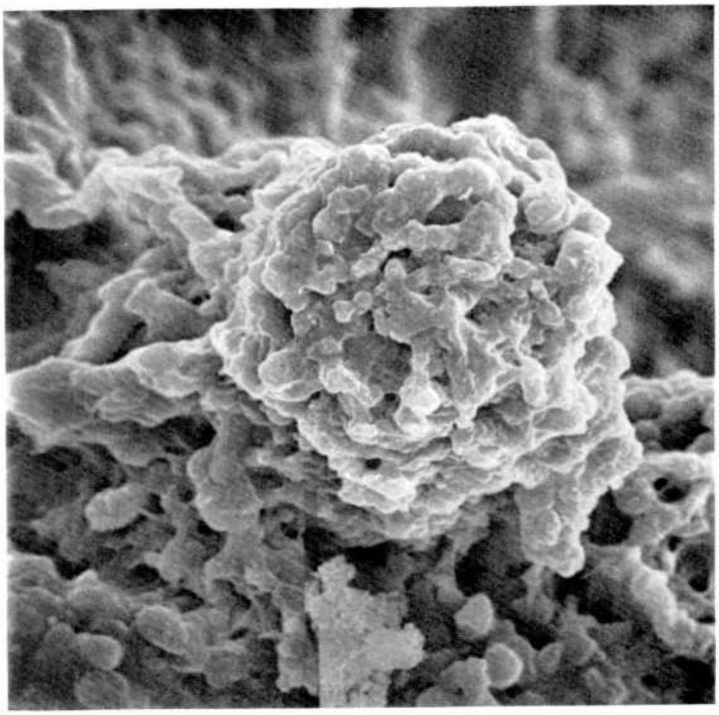

FIGURE 3. Madurella tissue grain showing laminated and polypoid surface. These structures were not destroyed by 50% KOH at 160°C for several hours (SEM, × 800).

in the instrument.

The standard electron microscope pictures showed the fungal hyphae, masses of interstitial debris, and a matrix which appeared from the striations to consist of fused and altered collagen fibrils (Fig. 4).

Experimental approach to grain production

On the evidence available to us the grain cement could most probably be composed of tissue components derived from the host, e.g. globulins and protein fibrils from the inflammatory granuloma such as collagen. Exposed to a polyphenol elaborated through the fungal enzyme systems and fungal metabolism, tanning of the protein by the polyphenol would account for the durability and resistance to the usual analytical procedures.

To induce a similar change experimentally, strips of rat tail collagen were placed in the solid medium near a growing *Madurella mycetomi* colony. The pigment progressively invaded and blackened the collagen strips. The strips grossly became jet black, and sections were difficult to cut because of extreme hardening with resulting shattering of the specimen. Cell material disappeared, and the collagen bundles were brown under the microscope. When globular protein was similarly treated (cooked egg-white) the pigment invaded the mass but did not adhere or alter its consistency. It was readily re-extracted.

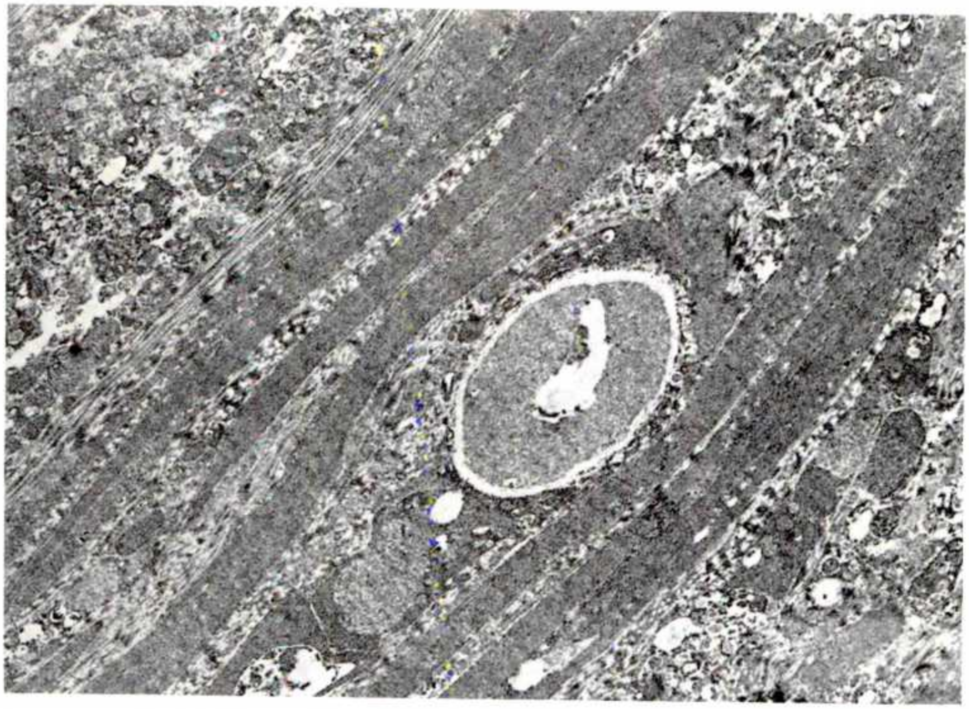

FIGURE 4. Fungal hypha flanked by somewhat dark granular strands of fibre bundles. Incorporated into the sides of these bundles are paler cross striated filaments considered to be collagen fibrils (× 17,420).

DISCUSSION

There can be little doubt that *Madurella mycetomi* in tissue of mycetoma produces several distinguishable effects. These would include pigment production, antibody responses, production of a semi-solid inflammatory response followed later by liquefaction and sinus formation, as well as necrotizing effects on cells, including allegedly the nerves. For a time, 6 months onward from the onset of infection, these results must follow in an intricate but reasonably constant pattern.

In the mature grain, it seems that the cement is formed by the slow tanning of those proteins deposited by living cells, the active inflammatory processes adjacent to the fungal cells, and a destruction of all host cells initially concerned herewith. The proteins available for tanning will obviously vary with the phase of the infection. The hollowing out of the centres of some grains may indicate that the grain nucleus as initially formed is less stable and may be composed of proteins which are not much hardened, e.g. immune complexes.

The fungus doubtless shelters in the grain rather as if it was living in a fortress, penetrable by nutrients only at the tips of the hyphae and possibly by only fairly small molecules. Drugs and antibodies of large molecular weight are quite possibly kept out of reach by the grain. Successful chemotherapy would seem to require a way to inhibit pigment production or perhaps the use of a low molecular weight fungicide.

ACKNOWLEDGMENTS

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Palmar and finger varicosities of the aged

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SUMMARY

A common abnormality of the veins of the fingers in elderly persons, with characteristic clinical features, is described. A survey shows that this is an age related condition. There was little evidence of an association with other abnormalities of the veins elsewhere. Histological findings were of dilated veins with thickened walls, and of larger circular thin walled lesions which were occasionally thrombosed.

Approximately half of the subjects surveyed showed evidence of these changes and there were no differences by sex. It seems that these lesions are properly included in the classification of the ageing phlebectasias and possible factors in their production are discussed.

Varicose abnormalities are predominantly seen in and associated with the lower extremity. Accounts of these lesions affecting the upper limb are confined, in the main, to isolated case reports of arteriovenous fistulae with marked dilatation and pulsation in young adults (Moore, 1964; Clarke, 1966; Nwafo, 1962). Bean (1958) stated that varicosities of the palms and fingers are very rare and that he had seen them only four times in his extensive experience, calling this condition palmar varix. Clark & Warren (1954) expressed the view that primary spontaneous varices of the upper extremity are seen so infrequently as to be curiosities. A recent review of the phlebectasias of old age (Kocsard, Ofner & D'Abrera, 1970) did not mention digital varicosities, and a single example of what appears to be this syndrome has been described by Johansen (1950). This present study describes a common abnormality of the digital and palmar veins in the aged, which does not seem to have received proper recognition in the literature.

THE LESIONS

These consist of blue dilated vessels which are situated over the palmar aspects of the digits and to a lesser extent over the distal part of the palms (Fig. 1). Minor examples or what appear to be early lesions are particularly marked in the digital flexor creases. The tips and the pulps of the fingers are not affected and the sides and dorsal aspects are rarely involved. They do not collapse when the hands are elevated but they can be 'milked' in the palmar direction (Fig. 2), and when emptied they are slow to refill remaining empty until the hands are lowered. They are best seen when the hands are warm.

The clinical picture is of tortuous and irregularly enlarged vessels of blue colour, associated with highly characteristic 'blob'-type lesions (Fig. 3). Sometimes they can be sufficiently large to elevate the overlying surface of the skin. Pressure with a slide empties them but manipulation is sometimes needed to empty the rounded blob lesions, leaving a small miliary spot. Some of these rounded lesions cannot be emptied and appear to be thrombosed, and in this instance they can be palpated in the skin. They transilluminate well and the blobs can be seen to be arising from the tortuous vessels.

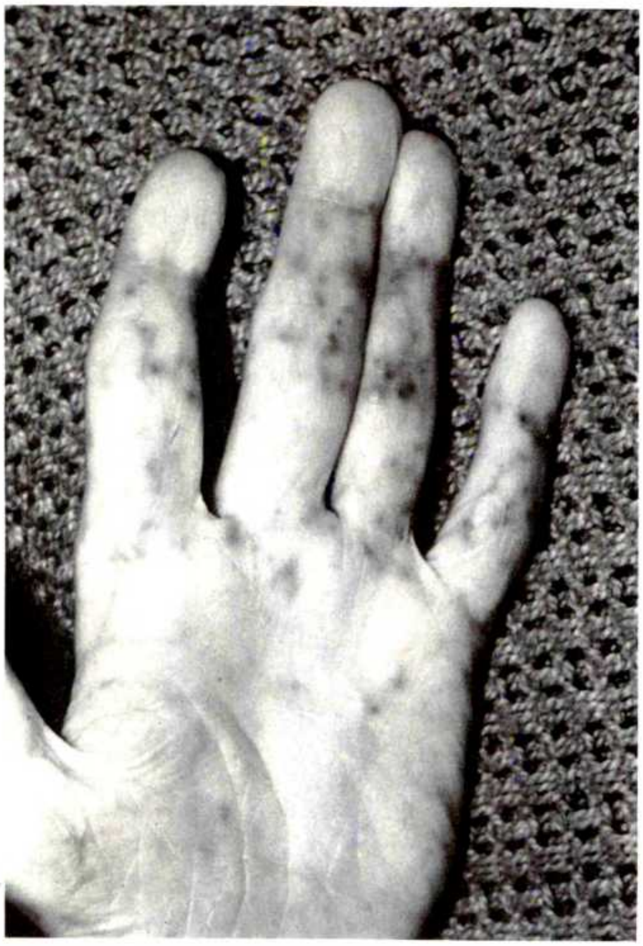

FIGURE 1. Typical appearances of the palmar aspect of the digits and palm (Stage 4).

Classification

As this syndrome became recognized it was possible to classify the appearances into four stages in terms of degree or severity (Fig. 4).

Stage 1. Small lesions in the finger creases only.

Stage 2. Lesions with extension onto the adjoining skin covering the phalanges.

Stage 3. More extensive lesions involving the length of the digits in two or more fingers of one hand.

Stage 4. Varicosities extending over the heads of the metacarpals and onto the palms.

The impression was that these stages were evolutionary and all examples with palmar lesions had Stage 3 lesions of the digits.

THE SURVEY

The hands of eighty-nine men and 327 women, who were inpatients in the Geriatric Unit, were inspected and the findings were classified according to stages 0–4. This survey yielded surprising results, with a little more than half (52%) showing these abnormalities, and sixty-eight patients (16%) had appearances of stage 3–4 varicosities.

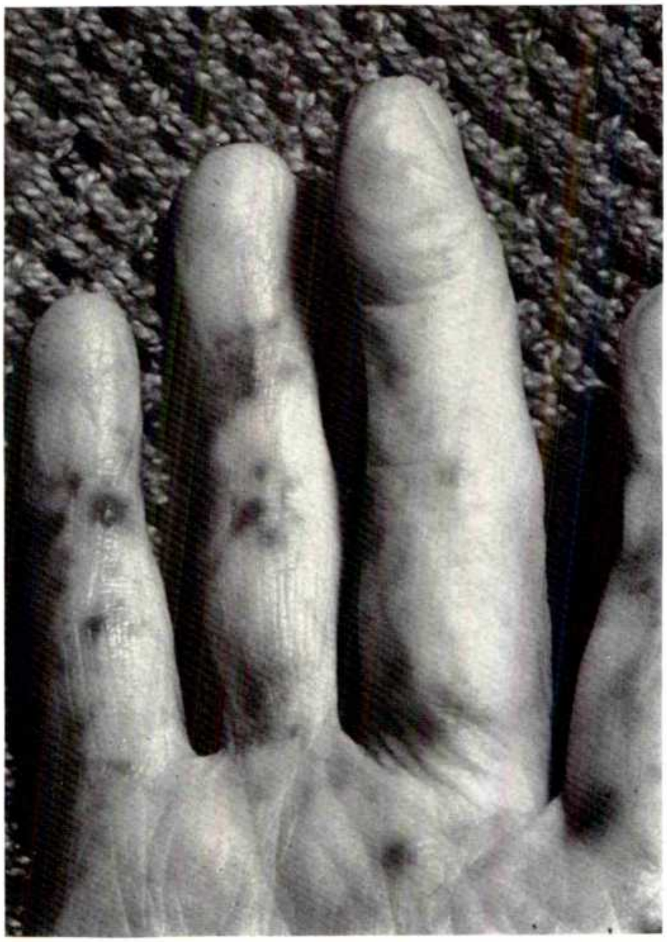

FIGURE 2. Lesions of the right hand showing distribution near the finger creases and absence in the pulps of the fingers. The middle finger has been 'milked' in the palmar direction.

The percentage incidence by sex is similar (Table 1), allowing for the fact that there were more women in the survey than men. Patients who were free from contractures and deformities and could have both hands inspected were examined for unilateral lesions, and these were found to be more common in the left hand than the right (Table 2). There was a significant tendency for the females to

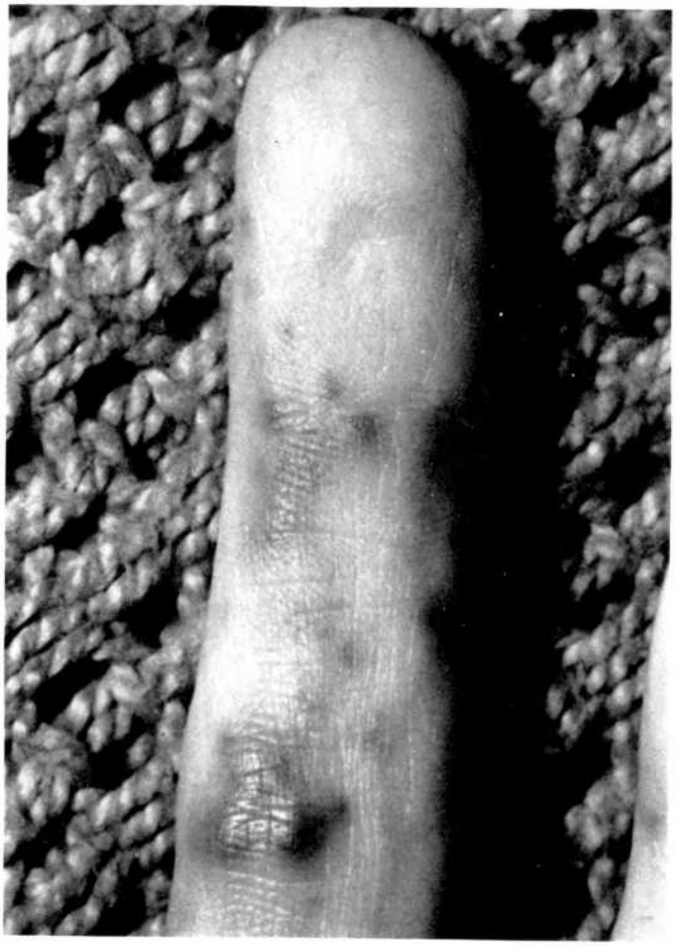

FIGURE 3. Lesions of left index finger showing characteristic appearances.

TABLE 1. Percentages by sex

	Lesions	Male (%)	Female (%)
	0	43	49
Stage	1,2	36	36
Stage	3,4	21	15

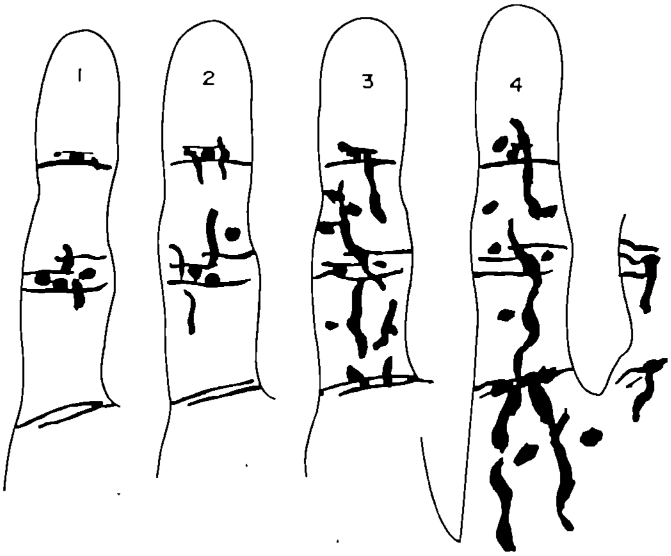

FIGURE 4. Grading of lesions in the survey.

TABLE 2. Unilateral lesions

	Male	Female
Right hand only	1	15
Left hand only	6	29

TABLE 3. Age, sex, and severity of lesions

Age (years)	Men			Women		
	0	1,2	3,4	0	1,2	3,4
Less than 50	—	—	—	4	1	—
50–59	5	2	—	4	—	—
60–69	9	3	0	16	6	2
70–79	14	14	9	38	31	12
80–89	6	11	9	72	63	24
90–99	4	2	1	26	15	11
100+	—	—	—	—	1	—

show lesions confined to the left hand only ($P < 0.05$), but there were insufficient numbers of men with unilateral lesions to demonstrate statistical significance.

Table 3 shows the incidence of lesions in relation to age by decade and sex, classified into: no lesions visible (0); mild lesions (stages 1 and 2); and marked lesions (stages 3 and 4). A statistical analysis was carried out on these data and it was found that there was no age × sex × lesion interaction and that the age relationship was the same for both sexes. There is an overall linear effect of age ($P < 0.01$) and the proportion of patients with no lesions falls steadily throughout the age groups.

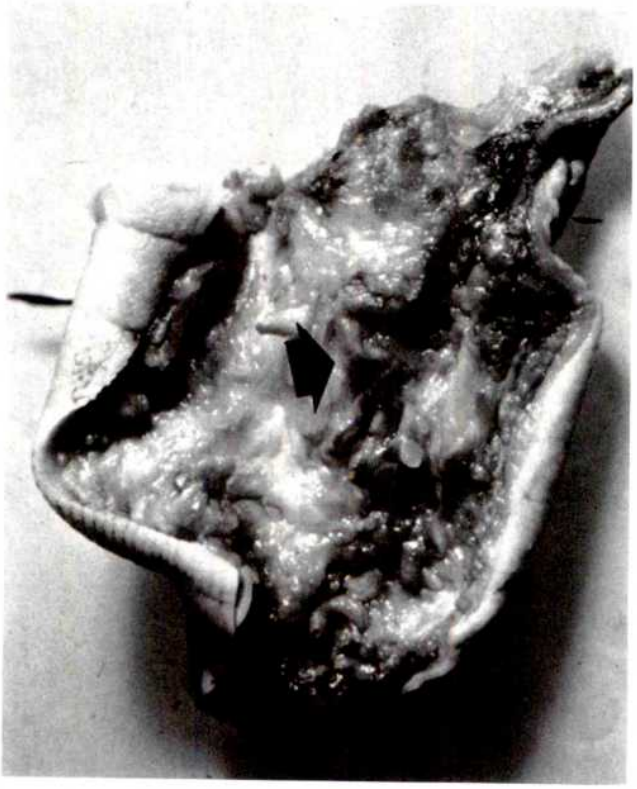

FIGURE 5. Macroscopic appearances showing tortuous thick walled vein in the palmar digital crease.

In examining this linear relationship, and when those with mild and marked lesions are grouped together, it is seen that the percentage with no lesions falls from 81% for those under 59 years to 42% for those in the 80–89 year group. The numbers with marked lesions were not sufficiently large to demonstrate a significantly consistent pattern, but this seems to grow with age.

Two patients were found who had haematomas of a digit which could have arisen from one of these lesions; otherwise they appeared to be benign and asymptomatic. There was no apparent association with phlebectasia elsewhere, in the mouth, on the lips, legs or scrotum, although occasional examples of these conditions were seen.

Lesions on the dorsa of the fingers were seen in two men and four women and these were confined to the dorsal aspect of the middle interphalangeal joints.

PATHOLOGY

Post mortem material was obtained from fourteen subjects who had well-marked lesions (stage 3–4) and from a similar number of controls who had normal appearances in life. Full thickness strips of digital palmar skin were removed from the palm to the finger tip and fixed in formol saline. Sections were stained with haematoxylin and eosin, elastic, fibrin and connective tissue stains.

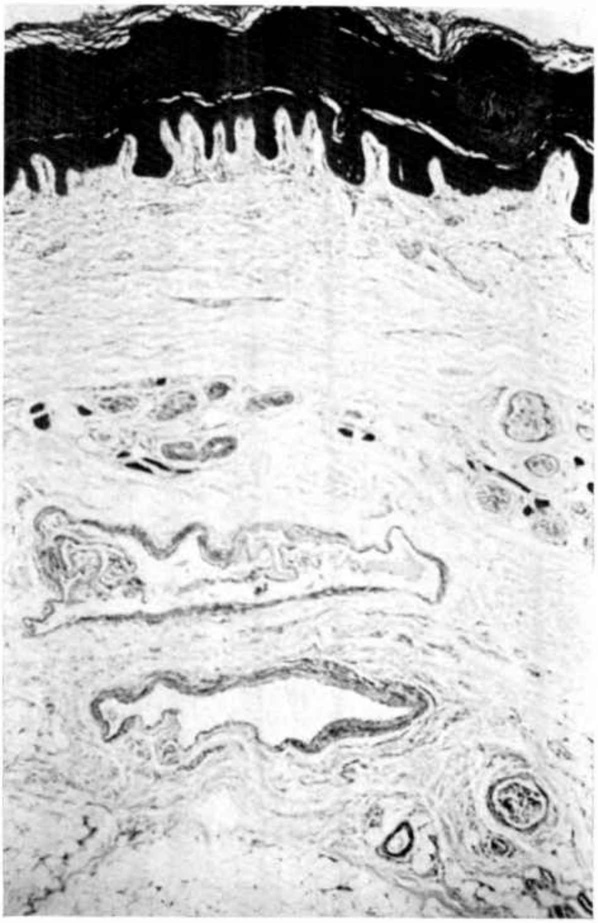

FIGURE 6. Abnormal palmar digital veins situated beneath the sweat glands (× 48).

There is little in the literature about the normal anatomy and histology of the veins of the fingers but they appear to be fairly narrow and thin walled structures situated mainly in the upper part of the dermis above the level of the sweat glands.

The patients who had lesions in life showed the following features.

(1) Tortuous veins which could for the most part be seen macroscopically (Fig. 5).

(2) Dilated thick walled veins which could be found at varying levels in the dermis and the subcutis (Fig. 6). For the most part the walls of these veins were of fairly uniform thickness due to an increase of the amount of smooth muscle in the media.

(3) Some veins showed marked dilatation and were very thin walled (Fig. 7), possibly showing 'blow-out' lesions, and this illustration shows a lesion which was thrombosed and could have caused a palpable nodule in the skin not disappearing on pressure.

(4) In some of the veins there was an appearance suggestive of valves (Fig. 6).

(5) There was no evidence of inflammation either in the walls or outside the vessels in any of the specimens examined.

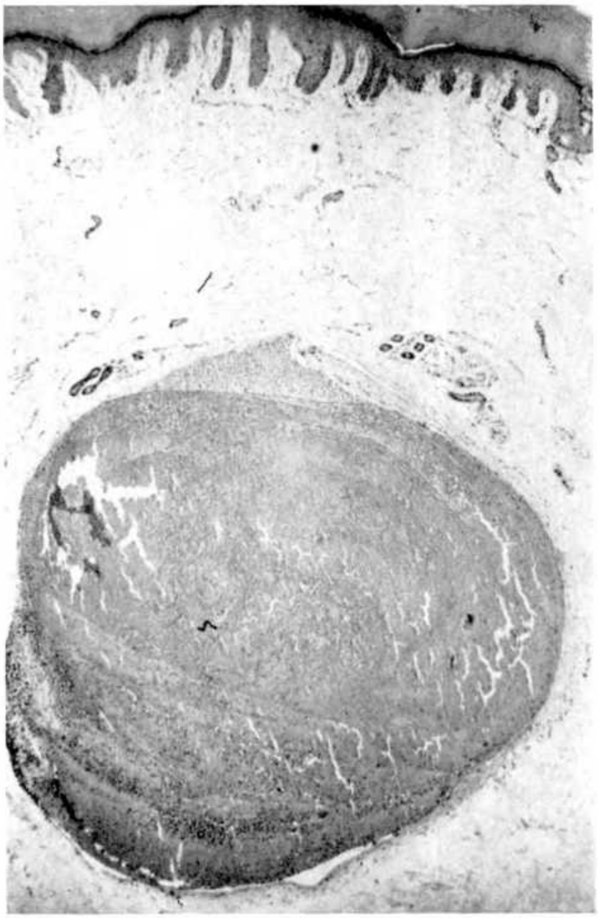

FIGURE 7. Large circular thin-walled thrombosed lesion (× 48).

DISCUSSION

This survey has demonstrated an abnormality of the palmar digital veins which appears in both sexes and shows a statistically significant increase with age. It would seem, therefore, that the increase with age relates to a specific ageing factor in causation.

The striking and distinct localization of these lesions to the palmar aspects of the fingers and especially to the areas of the digital flexor creases suggests that there is also a local factor.

The lesions causing this clinical appearance seem to be thick-walled veins which may have been subjected to venous hypertension for some time. The 'blob' lesions appear by their circular shape and greatly thinned walls to be of 'blow-out' type, but their presence suggests either a response to raised intraluminal pressure or to wall weakness, or to both of these factors. The extreme rarity of lesions over the dorsa of the fingers suggests that local factors are operating on the palmar surfaces of the hand and this could be due to mechanical compressive changes occurring during finger flexion.

Because the lesions appear to be almost always confined to the distal parts of the palms it would seem unlikely that they are caused by local obstructive factors above the level of the wrist. However, local pressure and obstruction at the metacarpophalangeal and interphalangeal creases is quite possible. It is clear that the process of gripping can cause intermittent compression of the palmar and digital veins. The absence of lesions over the dorsa of the fingers, except in the region of the middle interphalangeal joints, suggests that the site of causation of this process is almost entirely confined to the flexor aspects of the fingers. The dorsal aspect of the middle interphalangeal joint is the site of maximum flexion compared with the other joints involved in gripping. This mechanical factor may cause varying and intermittent increases in venous pressure with secondary wall hypertrophy.

Another possibility is that these veins are draining arteriovenous shunts or aneurysms. Such lesions have been identified around varicose veins and ulcers in the leg (Gius, 1960). Ryan (1973) believed that arteriovenous shunts often result from obstruction distal to the sites of arteriovenous anastomosis and are caused by increased venous pressure or stasis. Clinical observation does not suggest an increase of peripheral blood flow in our cases, and there is no clinical evidence of an increase in skin temperature of the digits. It would be difficult to explain why such lesions are confined to the flexor aspects of the fingers, and if arteriovenous aneurysms were demonstrated they could be secondary to venous obstruction rather than the primary cause of the clinical picture. Arteriovenous anastomoses are described as occurring in the extremities but they are said to be more marked in the finger tips, the nose and ears, and they tend to disappear with age (Gray's Anatomy, 1967).

There is evidence that degradation of connective tissue occurs with age and the thickening of the walls of the veins might be related, in part, to some loss of the supporting elements in the dermis. Tunbridge (1964) has described 'pseudo-elastin' formation in ageing skin, and particularly in senile and actinic elastosis elastic fibres seem excessive, but electron microscopy and biochemical tests show that this is abnormal and degenerate collagen formation (Hall, 1964). This has been described in skin exposed to ultraviolet radiation and in senile and steroid purpura. There was, however, no evidence of these changes or obvious microscopic evidence of excessive elastic tissue formation in our series of sections. Our findings tend to confirm those of Kocsard *et al.* (1970), who suggested that the failure of the ageing connective tissue to support the vessels is a probable cause of the phlebectatic lesions, and these changes are a local phenomenon due to locally acting factors. These lesions do not predict the existence of distant phlebectasias in other parts of the body.

It seems likely, therefore, that these palmar and finger varicosities fall within the classification of the ageing phlebectasias, in that this is an age-related condition where a combination of local, mechanical and collagen degradation factors are operating.

ACKNOWLEDGMENTS

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Elution of antibodies from the lymphocyte membrane in certain dermatoses

PRELIMINARY COMMUNICATION

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SUMMARY

The lymphocyte eluates of three patients with discoid lupus erythematosus and two patients with pemphigoid possessed basement membrane antibody of the IgG class either for normal human skin or guinea-pig lip.

The lymphocyte eluates from one patient with pemphigus did contain antibody (IgG) reactive with the intercellular substances of monkey oesophagus, and that of a patient with a drug reaction showed antibody reactive with basal cells of stratified epithelium.

These results indicate either antibody synthesis by the lymphocytes themselves, or that these cells may provide antigenic receptors reactive with specific antibody.

It is generally known that in pemphigus vulgaris the antibody is directed against the epidermal intercellular substances (Beutner & Jordon, 1964) and in pemphigoid against the basement membrane (Jordon *et al.*, 1967). In most instances these antibodies belong to the IgG class of immunoglobulins.

In lupus erythematosus no circulating antibody directed against specific skin antigens has been detected (Burnham, Neblett & Fine, 1963; Cormane, 1964). However, by means of acid eluate procedures, in addition to the nuclear antibody a basement membrane antibody that fixes complement and gives a linear immunofluorescence pattern, similar although not identical to the pemphigoid antibody, could be isolated from skin of patients with systemic lupus erythematosus (Beyvin & Thivolet, 1971; Landry & Sams, 1973).

Successful elution of skin fixed antibodies has also been performed in pemphigus vulgaris and pemphigoid (Thivolet, Beyvin & Le Mot, 1972).

The present study was undertaken to determine whether the aforementioned antibodies could be eluted from peripheral lymphocytes in three patients with discoid lupus erythematosus (LE); two patients with pemphigoid; one with pemphigus vulgaris and one with a drug reaction in which circulating antibody directed against the basal cells of stratified epithelium could be demonstrated (van Joost, 1974). In addition, circulating antibodies directed against the basal membrane of stratified epithelium could be demonstrated in the sera of the two patients with pemphigoid, and against the intercellular substances of monkey oesophagus in the serum of the one patient with pemphigus vulgaris, but not in the sera of the three patients with discoid LE.

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The lymphocytes were isolated from the peripheral blood according to the Isopaque–Ficoll technique (Böyum, 1968). The supernatants of the last washings of the isolated lymphocytes did not contain any demonstrable antibody. Subsequently, the acid elution procedure was performed as detailed by Feltkamp & Boode (1970) and the distribution pattern of immunoglobulin (Ig) and complement (C3) bearing lymphocytes assessed according to the counting chamber method as described in a previous paper (Cormane, Husz & Hamerlinck, 1974).

The lymphocyte eluates of the three patients with discoid LE and the two patients with pemphigoid possessed basement membrane antibody of the IgG class which reacted against either normal human skin or guinea-pig lip. In addition, one of the three discoid LE eluates had IgA antinuclear antibody.

The lymphocyte eluates from the patient with pemphigus did contain antibody (IgG) reactive with the intercellular substances of monkey oesophagus, and that from the patient with the drug reaction showed antibody reactive with basal cells of normal human skin (blood group O) or guinea-pig lip.

The distribution patterns of peripheral circulating lymphocytes carrying protein determinants showed an increase of the percentages of IgG and C3 bearing lymphocytes in the three patients with discoid LE, two patients with pemphigoid, one patient with pemphigus and one patient with a drug reaction. The percentages of lymphocytes bearing the other main classes of Ig were within the range of ten healthy controls, with one exception, the patient with the drug reaction, who also had an increased number of IgD and IgE bearing lymphocytes. Increased percentages of lymphocytes coated with IgD, IgE and IgG have been reported in drug reactions in a previous paper (Cormane, Hamerlinck & Husz, 1973).

These results suggest that sensitized circulating lymphocytes occur in patients with discoidlupus erythematosus, pemphigoid, pemphigus vulgaris and drug reactions. Moreover, they indicate that these cells may provide an antigenic receptor reactive with a specific antibody. Circulating antibody may not be detectable in the aforementioned diseases if it is bound by circulating lymphocytes and does not occur in excess.

The increased number of IgG bearing circulating lymphocytes in the patients under investigation can be explained by a reaction of the IgG antibody in question with the lymphocyte membrane. An alternative explanation could be a synthesis by the lymphocyte itself.

As the number of IgG and of C3 coated lymphocytes in one and the same patient was about the same, it seems very likely that both molecules occur concomitantly on the membrane of the lymphocyte. This would be consistent with the presence of immune complexes on the cell membrane and with the assumption that a reaction of the antibody with an antigenic determinant occurrs on the lymphocyte membrane.

Since, for example, the eluted intercellular substances antibody from the lymphocytes of the patient with pemphigus antibody had the same affinity for tissue antigen and gave the same pattern as pemphigus antibody, it seems very likely that these lymphocytes share common antigenic determinants with the specific antigenic sites of the skin. Further supportive evidence for a common antigenic determinant occurring on the membrane of the lymphocyte and in the intercellular substances of stratified epithelium is the finding of anti-epithelial antibodies of the pemphigus vulgaris type in heterologous antilymphocyte node sera (Grob & Inderbitzin, 1969) and antilymphocytic sera (Thivolet, Beyvin & André, 1970).

An analogous reasoning may pertain to the antigenic determinants occurring on the membranes of the IgG bearing lymphocytes of the three discoid LE and two pemphigoid patients which might also occur on the basement membrane of stratified epithelium. In the case of the patient with the drug reaction, the common antigenic determinant may be present in the cytoplasm and membrane of the basal cell layer as well.

We are now embarking on cross experiments with normal lymphocytes and pemphigoid serum.

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Pharmacology and Treatment

Tetracycline for the treatment of pityriasis lichenoides

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SUMMARY

Thirteen cases of pityriasis lichenoides were treated with oral tetracycline. Five of them were completely cured, another seven had to continue to take 1 g/day. Addition of an antihistamine in one patient who did not respond to tetracycline gave a dramatic result. Throat swab cultures yielded coagulase positive staphylococci in four cases and other kinds of bacteria in many other cases. Based on the results of the treatment and histological findings, it is postulated that the pathogenesis of pityriasis lichenoides may be due to a hypersensitivity reaction to infectious agents.

Spontaneous regression of pityriasis lichenoides (PL) may be seen in 3 months to a year. If the lesions do not subside in the first few years they tend to persist for many years afterwards (Marks, Black & Wilson Jones, 1972). Although many types of therapy have been suggested (Canizares, 1952; Winkelmann & Lorenc, 1959; Nagy & Balough, 1959; Verallo & Haserick, 1966; Shelley & Griffith, 1969; Tompkins, 1972; Zlatkov & Andreev, 1972; Harman, Nagington & Rook, 1972), none of these has been reported effective in a large number of cases. This paper records a number of cases which were treated successfully with oral tetracycline.

PATIENTS AND METHODS

Three males and ten females were seen at the Institute of Dermatology, Bangkok, Thailand, between August 1972 and July 1973. All the patients studied satisfied the criteria for diagnosis of Marks *et al.* (1972). Detailed history taking, physical examination and the following laboratory tests were performed: blood count, urine examination and culture, throat swab culture and ASO titre. The levels of immunoglobulins and complement were determined by single radial immunodiffusion plates. Skin tests, haemagglutination and fluorescence studies for toxoplasmosis were done. Skin biopsies were taken from early red papules (1 week old), scaly brownish papules (1–2 weeks old), chronic lesions (2 months old) and hypopigmented lesions. All patients had skin lesions which had not cleared before treatment started. Tetracycline 2 g/day was given to all patients, who were seen every week. If the lesions subsided a dosage of 1 g/day was given for a further month to prevent relapse. All patients were then re-examined after 6 months.

RESULTS

All patients had typical lesions of PL confirmed by histological examination. The histological picture was similar to that described by Marks & Black (1972). There were areas of focal parakeratosis, inter- and intra-cellular oedema, and benign dyskeratotic cells within the epidermis. The dermal papillae showed oedema with dilatation of superficial vessels. There was extravasation of red cells and perivascular infiltration with histiocytes and lymphocytes. Invasion of the epidermis by red cells was seen in haemorrhagic lesions. There was no true vasculitis in acute lesions; fibrinoid change, necrosis of the blood vessels and nuclear dust were not present. Non-specific dermatitis was seen in chronic or healing lesions.

Throat swab cultures revealed coagulase positive staphylococci in four cases whose tonsillar areas were slightly inflamed. Skin tests, haemagglutination and fluorescence studies for toxoplasmosis were negative. Immunoglobulin levels were within normal limits. The result s of other laboratory tests were also within normal limits.

Remission occurred in five patients (see Table 1, cases 1–5); the lesions disappeared in 2–4 weeks and there was no recurrence in a 6-month period. Another seven patients (cases 6–12) also responded within 4 weeks but they had to continue with at least 1 g/day of tetracycline as this appeared to keep the lesions under control. Two chronic cases (cases 9 and 10) admitted that this was the first time that their skin was clear, although a few new red papules reappeared even when they were on 1 g daily. So far there have been no side effects from treatment. Case 13, with the clinical picture of Mucha–Habermann disease, did not respond. New crops of red papules were seen even though the patient was on 2 g/day during the 6 month course of treatment; when chlorpheniramine maleate 12 mg/day was added, all the lesions regressed in 4 weeks.

DISCUSSION

Marks *et al.* (1972) recently presented clinical evidence to suggest that pityriasis lichenoides et varioliformis acuta (PLVA) and pityriasis lichenoides chronica are merely variants of a single disease process. They were not in favour of Szymanski's concept of vasculitis (1959) as the cause of PLVA. Pinkus & Mehregan (1969) also felt that PLVA and PLC were the same disease. Many treatments have been used, and more recently tetracycline was reported to be effective in controlling the disease (Shelley & Griffith, 1969; Tompkins, 1972). In this study, since throat swab cultures yielded coagulase positive staphylococci in four cases, and other kinds of bacteria and normal flora in several cases, tetracycline was tried and the results were quite successful. An antihistamine was added in case 13, who did not respond to the prolonged course of tetracycline. Interestingly enough, a dramatic response was seen in 4 weeks. Whether this combined treatment is useful is still under investigation.

It is probable that this disease is the result of a hypersensitivity reaction to infectious agents that cause a release of vasoactive substances. Toxoplasma infection was excluded by the absence of positive laboratory tests in this study as it was in the study of Marks *et al.* (1972). This contrasts with the hypothesis of Zlatkov & Andreev (1972). Although the aetiology is still unknown, tetracycline is safer than either methotrexate (Cornelison, Knox & Everett, 1972) or systemic corticosteroids.

ACKNOWLEDGMENTS

The author would like to thank Professor W.B.Shelley, Philadelphia, for his helpful criticism and Dr Vichai Sangkasuwan, SEATO Laboratory, Bangkok, for the toxoplasmosis study.

TABLE 1.

Case	Sex	Age (years)	Duration before treatment	Time to onset of improvement	Complete involution* within	Throat swab culture	Result at 6 months follow-up
1	F	24	2 months	1 week	3 weeks	Not done	No recurrence
2	F	20	5 months	1 month	3 months	Not done	No recurrence
3	M	54	3 months	1 week	3 weeks	Not done	No recurrence
4	M	21	2 years	1 week	10 days	*Diplococcus pneumoniae*	No recurrence
5	F	29	10 years	2 weeks	1 month	Coagulase positive staph., β strep. not group A	No recurrence
6	F	16	2 years	1 week	—	Coagulase positive staph.	No lesions, patient still on 1 g/day
7	F	30	8 months	1 week	3 weeks	No growth	Recurred in 3 months and again controlled by tetracycline 1 g/day
8	M	11	6 months	1 month	—	Coagulase positive staph.	No lesions while on 2 g/day
9	F	22	10 years	1 month	—	Coagulase positive staph.	Few lesions; patient still on 1 g/day
10	F	18	17 years	1 month	—	No growth	Few lesions; patient still on 1 g/day
11	F	20	1 year	2 weeks	—	Coagulase negative staph.	Recurred when no tetracycline was given; patient still on 1 g/day
12	F	19	2 years	2 weeks	—	*Pseudomonas aeruginosa* β strep. group A	No lesions; patient still on 1 g/day
13	F	19	6 months	—	—	Aerobacter	No response in 6 months then chlorpheniramine was added causing prompt response

* Tetracycline dosage reduced to 1 g daily and discontinued 1 month after without flare-up.

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Comparative bio-availability of proprietary topical corticosteroid preparations; vasoconstrictor assays on thirty creams and gels

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SUMMARY

Thirty proprietary corticosteroid creams and gels were evaluated for vasoconstriction using an occluded blanching test. The method differed from previous investigators' in employing a more sensitive estimation of pallor and more reading times in order to determine the complete blanching profile. The results were expressed as three parameters: (1) area under the blanching curve, (2) summed % total possible score, and (3) square root transformation of sum of scores divided by number of volunteers; the last permitted statistical differentiation between the preparations. Experiments were performed to (a) compare the bio-availability of the formulations, (b) determine the effect of antimicrobial additives, and (c) assess the retention of steroids in the skin. Scores for the additive-containing and 'plain' preparations were within 90% except for Tri-Adcortyl/Adcortyl (78%) and Locorten–Vioform/Locorten (53%). A steroid reservoir in the skin was demonstrated for up to 14 days. Selected preparations were re-tested by a non-occluded method and the results compared with those obtained by the occluded procedure.

Corticosteroid preparations are widely used in dermatology and during the last 15 years they have revolutionized the treatment of many inflammatory skin diseases. Research departments of pharmaceutical companies continue to develop more potent steroids in improved bases for topical application. Concomitant with that work has been the development of *in vitro* and *in vivo* techniques for evaluating the activity of corticosteroids and their formulations. Recent bio-assay systems used for this purpose include modifications of the McKenzie & Stoughton blanching test (Christie & Moore-Robinson, 1970; Place, Velazquez & Burdick, 1970; Falconi & Rossi, 1972), the psoriasis assay (Dumas & Scholtz, 1972), the suppression of ultraviolet light-induced erythema (Burdick *et al.*, 1973), and the anti-mitotic effect on stripped skin of the hairless mouse (Marks, Pongsehirun & Saylan, 1973). Irrespective of which bio-assay is used, it is customary to evaluate corticosteroid preparations in clinical trial and reports continue to appear concerning steroid-containing lotions (Harris, 1972; Neering, van der Kroon & Roeleveld, 1972), creams (Mackey, 1973; Gentry, Rosenberg & Goltz, 1973; Polano & Kanaar, 1973), and gels (Cullen, 1973).

Clinical trials do not permit the comparison of more than a few preparations at the same time. In these cases, where the use of many formulations for simultaneous evaluation is limited, an incomplete picture may arise concerning the relative value of the preparations. This difficulty may be overcome by evaluating several products by the vasoconstrictor test in healthy volunteers (Coldman, Lockerbie & Laws, 1971). A relationship has been demonstrated between the ability of topically applied steroids to produce blanching and to alleviate inflammation in use (Place *et al.*, 1970). Stoughton (1972) found that the results of his blanching test using commercial preparations correlated well with clinical studies and he suggested that the vasoconstrictor assay can predict the clinical effectiveness of a given formulation.

Although two recent publications (Coldman *et al.*, 1971; Stoughton, 1972) were concerned with the vasoconstrictor response to proprietary corticosteroid preparations, in neither case was an extensive range of products investigated nor did the authors consider the effect of other factors on the results obtained. The work reported in the present paper was performed in order to (a) compare the skin blanching activity and, by inference, the bio-availability of proprietary hydrophilic topical steroid preparations; (b) determine the effect of antimicrobial additives on the blanching response; (c) assess the retention of steroids in the skin; and (d) investigate the effect of test conditions. The term 'bio-availability' of the products described in this paper refers to the release of the steroids from the preparation followed by its penetration of the epidermis to produce the characteristic vasoconstrictor effect. The present work differs from previous studies involving proprietary formulations in employing (a) a comprehensive range of hydrophilic products; (b) a more sensitive assessment of pallor; and (c) more reading times in order to determine the complete blanching profile from which more significant conclusions may be drawn.

EXPERIMENTAL

Corticosteroid formulations

Commercial packs of thirty hydrophilic topical steroid formulations (Table 1) were stored at room temperature for not less than 2 weeks and the first gram of product obtained from each tube was rejected.

Volunteers

Ten volunteers were selected from an initial screening of eighteen persons as those demonstrating consistency of response to a standard preparation (Betnovate Cream) but without reference to their sex or steroid sensitivity. None had received topical application of corticosteroids for at least 3 months prior to the investigations and an interval of 2 months was allowed between trials.

Methods

The blanching test employed was a modification of that described by Christie & Moore-Robinson (1970) and incorporated a double-blind technique. 5 ± 1 mg of thirty proprietary preparations were applied in three trials to the washed flexor surfaces of both fore-arms, preliminary work having shown that variations within the range 3–8 mg did not significantly affect the degree of pallor produced. The applications sites consisted of 7 × 7 mm areas punched out from Blenderm polyethylene tape (3M Medical Products, London) to which the formulations were applied by reference to previously prepared randomization sheets; a total of twenty sites were used for each preparation. The sites were occluded with type S12 μm Melinex polyester film (ICI Plastics Division, Welwyn Garden City) for 6 h. The areas were washed with soap and water at skin temperature, were dried, and the degree of pallor was estimated 10 min later using a 0–4 scale with half-point ratings based on that of Pepler,

TABLE 1. Proprietary preparations used in vasoconstrictor assays

Preparation	Manufacturer	Steroid and % w/w	Additive and % w/w
Adcortyl Cream	Squibb	Triamcinolone acetonide, 0·1	—
Betnovate Cream	Glaxo	Betamethasone as valerate, 0·1	—
Betnovate-C Cream	Glaxo	Betamethasone as valerate, 0·1	Clioquinol, 3·0
Betnovate-N Cream	Glaxo	Betamethasone as valerate, 0·1	Neomycin sulphate, 0·5
Deflamene Cream	Pharmitalia	Formocortol, 0·025	—
Deflamene-A Cream	Pharmitalia	Formocortol, 0·025	Paromomycin sulphate, 0·5
Dermovate Cream	Glaxo	Clobetasol propionate, 0·05	—
Drenison Cream	Lilly	Flurandrenolone, 0·05	—
Drenison with Neomycin Cream	Lilly	Flurandrenolone, 0·05	Neomycin sulphate, 0·5
Ficoid-2 Cream	Fisons	Fluocortolone pivalate, 0·1 Fluocortolone hexanoate, 0·1	—
Haelan Cream	Dista	Flurandrenolone, 0·0125	—
Haelan-C Cream	Dista	Flurandrenolone, 0·0125	Clioquinol, 3
Hydrocortistab Cream	Boots	Hydrocortisone acetate, 1·0	—
Hydrocortisyl Cream	Roussel	Hydrocortisone, 1·0	—
Ledercort Cream	Lederle	Triamcinolone acetonide, 0·1	—
Ledercort with Neomycin Cream	Lederle	Triamcinolone acetonide, 0·1	Neomycin sulphate, 0·5
Locorten Cream	Ciba	Flumethasone pivalate, 0·02	—
Locorten-Vioform Cream	Ciba	Flumethasone pivalate, 0·02	Clioquinol, 3
Metosyn	ICI	Fluocinonide, 0·05	—
Propaderm Cream	Allen & Hanburys	Beclomethasone dipropionate, 0·025	—
Propaderm-C Cream	Allen & Hanburys	Beclomethasone dipropionate, 0·025	Clioquinol, 3
Propaderm-N Cream	Allen & Hanburys	Beclomethasone dipropionate, 0·025	Neomycin sulphate, 0·5
Synalar Cream	ICI	Fluocinolone acetonide, 0·025	—
Synalar-C Cream	ICI	Fluocinolone acetonide, 0·025	Clioquinol, 3
Synalar-N Cream	ICI	Fluocinolone acetonide, 0·025	Neomycin sulphate, 0·5
Synalar Gel	ICI	Fluocinolone acetonide, 0·025	—
Topilar Cream	Syntex	Fluclorolone acetonide, 0·025	—
Tri-Adcortyl Cream	Squibb	Triamcinolone acetonide, 0·1	Neomycin (as sulphate), 0·25 Gramicidin, 0·025 Nystatin, 100,000 iu/g
Tridesilon Cream	Dome	Desonide 0·05	—
Ultralanum Cream	Schering	Fluocortolone pivalate, 0·25 Fluocortolone hexanoate, 0·25	—

Woodford & Morrison (1971): 0, normal skin; 1, slight pallor of indistinct outline; 2, pallor with at least two corners outlined; 3, even pallor with a clear outline of the square; 4, very intense pallor.

Readings were taken after a further 1, 2, 3, 6, 18, 26, 42, 66, 74 and 90 h to provide data points for skin blanching at 6, 7, 8, 9, 12, 24, 32, 48, 72, 80 and 96 h after application. All estimations of pallor were performed in a double-blind manner without reference to the application charts. Throughout the duration of the tests the volunteers avoided elevated temperatures (environmental or those produced by exercise) and did not allow their arms to come into contact with water.

Selected preparations were re-tested by a non-occluded method in which the Blenderm tape was removed after application of the formulations and the Melinex film was replaced by a perforated metal screen.

In order to assess the retention of steroid in the skin the sites in selected volunteers were re-occluded with Melinex film for 12 h, 8, 12 and 14 days after the commencement of the experiment. Pallor was estimated 2 h later, when blanching was maximal.

RESULTS AND DISCUSSION

Previous investigators employing the blanching test have recorded the presence or absence of pallor (Place *et al.*, 1970) or have estimated the degree of vasoconstriction produced, either on a 0–3 (Stoughton, 1972) or 0–4 scale (Pepler *et al.*, 1971). Similarly, the number of reading times has varied from a single occasion (Falconi & Rossi, 1972) to four over a 48-h period (Coldman *et al.*, 1971). In the present work the number of reading times was extended until no pallor was visible in order to obtain the complete blanching profile, and a half-point rating scale provided more accurate differentiation between preparations.

Occluded test

With each preparation the results for all volunteers were expressed as a percentage of the total possible score at each time period. The method of calculation was as follows: maximum score per site = 4; for two arms, $4 \times 2 = 8$; for ten volunteers, $8 \times 10 = 80$ (e.g. 6 h reading for Synalar Cream, score of 41 out of a possible 80 = 51.3%). Betnovate Cream was employed in each of the three trials to detect any variation in the volunteers' responses to a 'standard' preparation. Statistical analysis using the F-test (Goldstein, 1964) showed that there was no significant difference ($P < 0{\cdot}05$) between the results obtained with Betnovate Cream in each trial. However, the possibility exists that in future experiments there may be a significant difference in the control Betnovate result because of, for example, change in volunteer panel composition, alteration in environmental conditions, etc. To allow for this possibility a method of unification of data was evolved, as follows the percentages of the total possible score values for all preparations in each trial were multiplied by the value: mean total value for Betnovate Cream for all three trials/total value for Betnovate Cream in that particular trial. By this means the results for all preparations employed in the three separate trials were comparable with each other and could be related to subsequent investigations. Blanching profiles for all thirty preparations are shown in Figs. 1–7, separated and occasionally dotted for clarity and ease of discussion. Experimental points were omitted, but all lay on or near the curves as drawn. Nearly all the preparations gave curves with a peak in the 9–12 h region with a marked fall-off thereafter, notable exceptions being Hydrocortisyl (where the scores decreased continuously after 6 h: Fig. 7) and Synalar Gel and Ledercort (where the 32 h responses were more than 70% of the peak values; Figs. 4 and 5).

In most cases antimicrobial additions did not markedly affect the shape of the curve (although the additive-containing products provided profiles with sharper peaks) or the percentage of the total

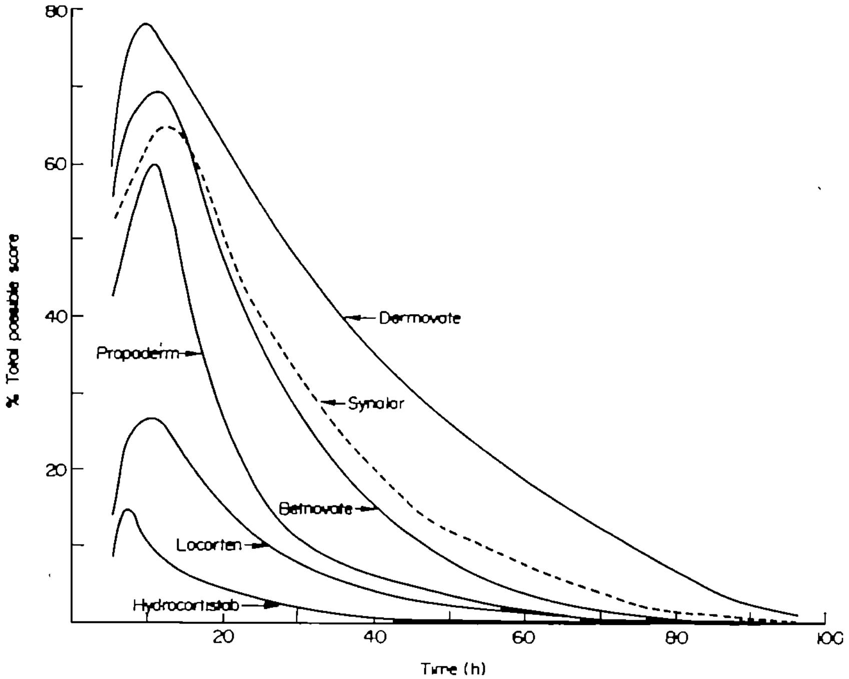

FIGURE 1. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

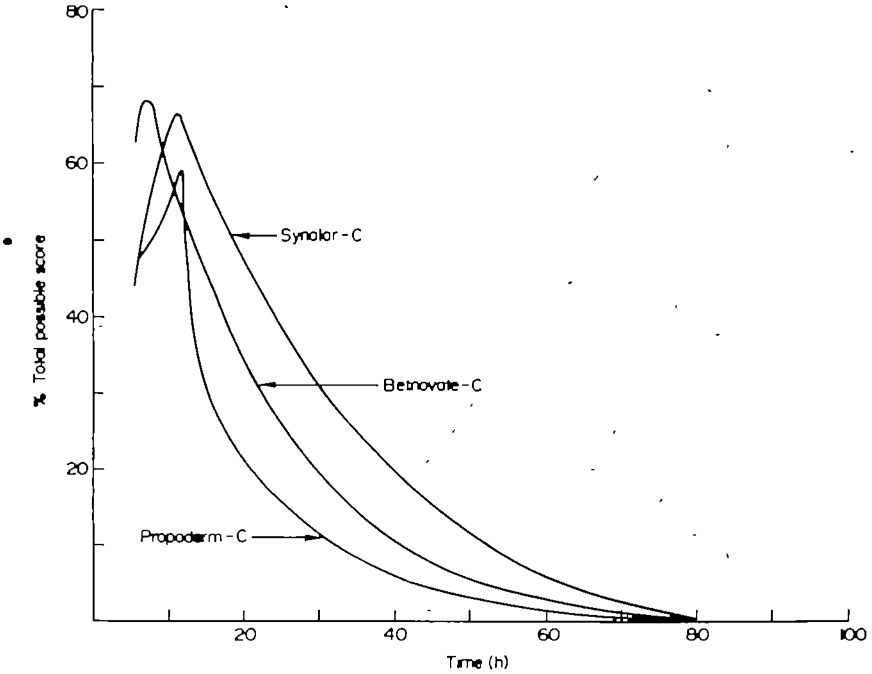

FIGURE 2. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

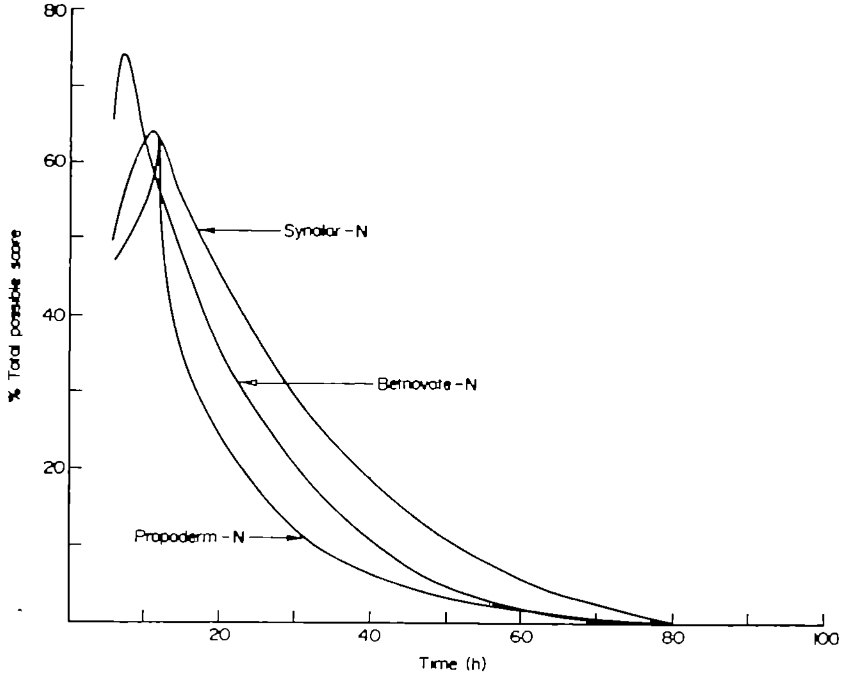

FIGURE 3. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

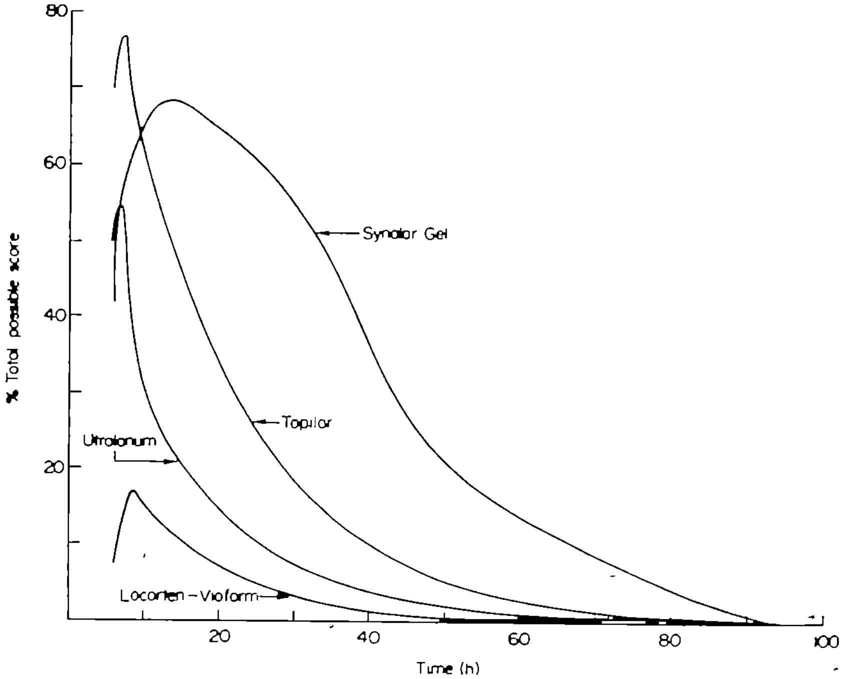

FIGURE 4. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

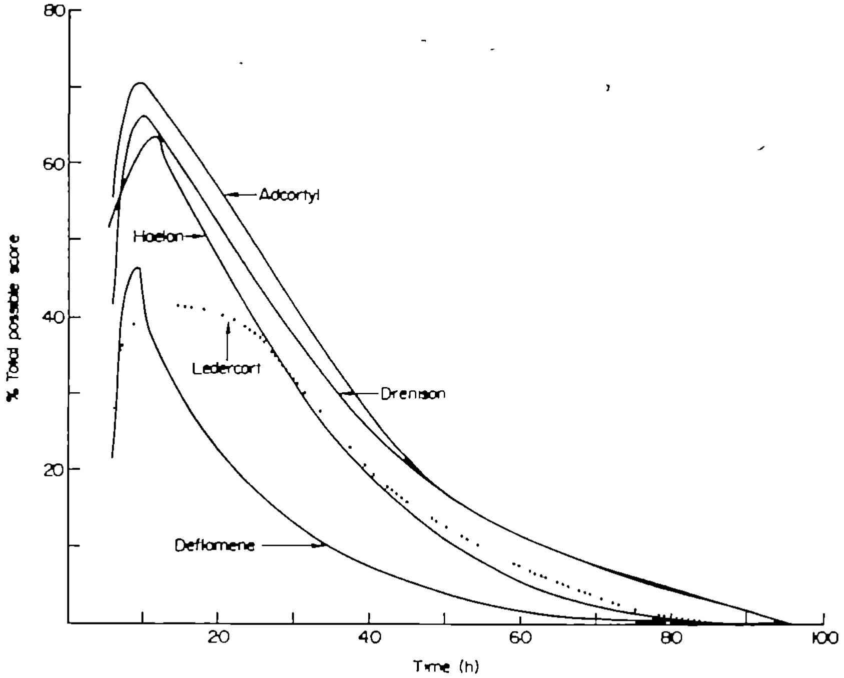

FIGURE 5. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

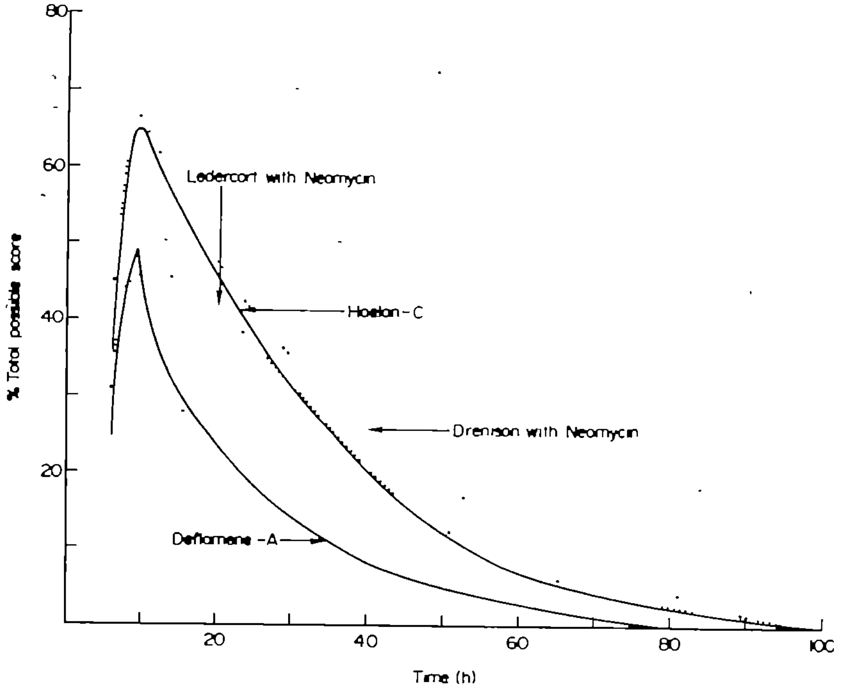

FIGURE 6. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

TABLE 2. Blanching response to thirty topical corticosteroid preparations, arranged in rank order of area under the curve values

Preparation	Blanching response*						
	Area under the curve %×h†		Summed % total possible score‡		Tm/10 mean value, square root trans-formation§	% Total possible score on re-occlusion**	
	Occluded	Non-occluded	Occluded	Non-occluded	Occluded	Occluded	Non-occluded
Dermovate Cream	2800	—	512	—	6·35	50	—
Synalar Gel	2630	—	440	—	5·91	23	—
Adcortyl Cream	2280	—	442	—	5·92	15	—
Drenison Cream	2150	—	397	—	5·63	15	—
Drenison with Neomycin Cream	2060	—	399	—	5·63	10	—
Metosyn	1960	—	409	—	5·70	21	—
Tridesilon Cream	1860	—	373	—	5·44	6	—
Synalar Cream	1850	1830	391	276	5·57	15	8
Haelan-C Cream	1810	—	362	—	5·36	8	—
Tri-Adcortyl Cream	1780	—	342	—	5·23	13	—
Synalar-C Cream	1780	1710	362	249	5·34	8	4
Synalar-N Cream	1730	1710	369	261	5·39	15	4
Haelan Cream	1720	—	372	—	5·43	8	—
Betnovate Cream	1670	1060	393	273	5·60	14	10
Ledercort with Neomycin Cream	1600	—	297	—	4·82	4	—
Ledercort Cream	1530	—	271	—	4·61	6	—
Betnovate-N Cream	1300	1130	385	291	5·52	10	4
Topilar Cream	1270	1720	393	323	5·58	8	6
Betnovate-C Cream	1210	1220	355	284	5·30	6	6
Propaderm Cream	1000	546	293	150	4·75	4	0
Propaderm-N Cream	960	518	297	160	4·84	4	0
Propaderm-C Cream	920	537	290	152	4·77	4	0
Deflamene-A Cream	900	—	230	—	4·26	0	—
Deflamene Cream	840	—	216	—	4·13	0	—
Ultralanum Cream	596	—	232	—	4·23	4	—

Locorten Cream	545	310	136	76·3	3·22	0	0
Ficoid-2 Cream	493	—	239	—	4·33	6	—
Locorten-Vioform Cream	257	177	71·7	49·4	2·33	0	0
Hydrocortisyl Cream	180	—	59·2	—	2·13	0	—
Hydrocortistab Cream	167	—	61·7	—	2·03	0	—

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* Values quoted to three significant figures for data based on full panel of ten volunteers.

† Obtained by planimetry of the blanching profile.

‡ The percentage of the total possible scores summed for all volunteers over all reading times.

§ The Tm/10 value is the square root transformation of sum of scores (Tm) divided by the number of volunteers (10). The minimum significant range value k = 0·87 ($P < 0{\cdot}05$), i.e. if the Tm/10 values of two preparations differ by more than 0·87 there is a significant difference between those preparations.

** Re-occluded for 12 h, 8 days after commencement of experiment: three volunteers (statistical treatment inapplicable).

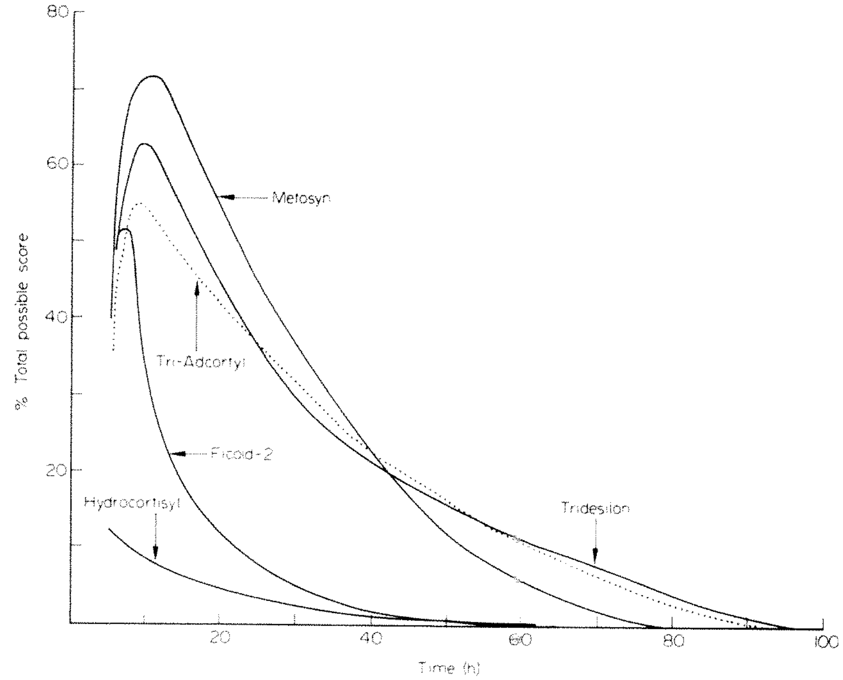

FIGURE 7. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

possible score. The summed percentage of the total possible scores (i.e. the percentage of the total possible scores summed for all time periods) for the 'plain' and corresponding additive-containing preparations were within 90%, except for Tri-Adcortyl and Locorten–Vioform which were 78% and 53% that of their respective 'plain' formulations (Table 2). This depression of score was further checked for Locorten/Locorten–Vioform preparations in a further full trial and essentially the same result was obtained. The diminution in blanching response to Tri-Adcortyl and Locorten–Vioform may be due to decreased availability of the steroid arising from (a) interaction between the steroid and antimicrobial compound, (b) difference in the composition of the cream bases in the presence and absence of antimicrobial additive, or (c) a combination of these two effects. Such factors would affect the thermodynamic activity of the steroid and hence its release from the base and subsequent penetration into the skin. With both preparations the compositions of the bases were complex and differed according to the presence or absence of antimicrobial additives. Fundamental physico-chemical studies are necessary to determine the precise reason for this diminution in blanching response. Raab & Windisch (1973) found that gentamicin did not significantly affect the amount of pallor produced by betamethasone dipropionate when both compounds were present in aqueous–alcoholic solution. However, there is little information concerning the effect of antibiotics on blanching produced by steroid-containing creams.

The area under the curves in Figs 1–7 was obtained by planimetry (Allbrit fixed index planimeter, short arm Shandon model; W.F. Stanley, New Eltham, London) and expressed in units of 'percentage of the total possible score × h'. Table 2 shows the proprietary formulations arranged in decreasing order of area under the curve values, facilitating direct comparison of preparations using a biological response–time parameter. It is interesting to note that the order is not exactly the same as that given by a summed percentage of the total possible score classification. Curves showing sharp peaks followed by a rapid decline (e.g. Ficoid-2, Fig. 7) gave modest area under the curve values. However, graphs exhibiting a flatter maximum with a more gradual fall-off (e.g. Locorten, Fig. 1) produced greater area under the curve values although the sum of their percentage of the total possible scores at the reading times employed may be less. Because of the analogy with blood concentration–time curves for oral and parenteral preparations, it is suggested that comparison of topical steroid preparations by their true area under the curve values is the preferred method, although it is not readily amenable to statistical analysis.

The blanching results were also arranged in a manner suitable for statistical treatment, as follows. The scores for each volunteer were summed over all reading times for each preparation and subjected to an analysis of variance using an Elliott 803B computer. The computer programme was arranged so that the data could be analysed without transformation or in one of five (x^{-1}, $x^{-\frac{1}{2}}$, log x, $x^{\frac{1}{2}}$ and x^2) transformations (Tukey, 1957). Tests for non-additivity (Harter & Lum, 1962) indicated a preference for the square root transformation and the analysis of variance was calculated using those values. The results of the variance analysis showed that, for the three occluded trials treated as one experiment, statistically highly significant differences existed between the preparations ($F = 47{\cdot}76$, $P<0{\cdot}01$) and between the volunteers ($F = 12{\cdot}25$, $P<0{\cdot}01$). Calculation of the minimum significant range value k using the Studentized range test (Goldstein, 1964) permitted comparison of the proprietary formulations. Significant differences between preparations occurred when their Tm/10 mean values (Table 2) differed by a figure greater than the value of k. For example, the non-significance range for Propaderm was 4·75±0·87, i.e. 5·62–3·88. Hence, Propaderm was not significantly different to all preparations in Table 2 from Tridesilon to Ficoid-2 inclusive, with the exception of Locorten (Tm/10 = 3·22), which gave a significantly lower blanching response. Propaderm was significantly poorer than the first six preparations in Table 2 but significantly better than the final three formulations. The reason for the apparent lack of statistical difference between many preparations was partly due to the variation between volunteers—with most preparations the total score for all time periods given by the 'most sensitive' subject was approximately twice that produced by the 'least sensitive' volunteer.

Adcortyl and Ledercort contain the same percentage of the same steroid. However, the Adcortyl mean value (Tm/10) was significantly greater than that for Ledercort ($P<0{\cdot}05$) while the area under the curve and summed percentage of the total possible score values for Adcortyl were approximately 150% and 160%, respectively, those of Ledercort (Table 2). These results, together with consideration of blanching profiles (Fig. 5), suggest that the steroid in Adcortyl has a higher bio-availability than that in Ledercort. This may be due to more of the steroid being in solution or in a more favourable particle size, polymorphic form, cream base, etc.

Two other pairs of preparations contained the same steroid or steroids, but in different concentrations (Drenison and Haelan, Ultralanum and Ficoid-2, Table 1). Within neither pair was there a significant difference between mean values but the stronger preparations of each pair (Drenison and Ultralanum) gave greater area under the curve values (Table 2). Both preparations in each pair exhibited similar blanching profiles (Drenison and Haelan, Fig. 5; Ultralanum and Ficoid-2, Figs. 4 and 7).

Re-occlusion of the application sites in only three volunteers (for which, therefore, statistical analysis was inapplicable) 8 days after commencement of the experiment permitted estimation of steroid retention in the stratum corneum (Vickers, 1963). Preliminary experiments showed that an increased re-occlusion time (12 h) facilitated estimation of pallor and that blanching was maximal 2 h after removal of the Melinex film, pallor usually disappearing within a further 10 h. The degree of maximum blanching was, in general, proportional to the ranking of preparations (Table 2). The percentage of the total possible score for Dermovate (50) was approximately twice that of Synalar Gel (23) and Metosyn (21), while most of the preparations from Adcortyl down to Topilar gave values of

15–8. Lower scores were produced by Tridesilon, the Ledercort and Propaderm formulations, Ultralanum and Ficoid-2, while for the remainder (Deflamene and Locorten preparations, Hydrocortisyl and Hydrocortistab) no pallor was visible at the application site. Subsequent re-occlusion showed that the presence of steroids in the skin could not be demonstrated after 12 days, with the exception of Dermovate which apparently disappeared after 14 days. Vickers (1963) found that the presence of potent steroids applied in alcoholic solution could be demonstrated for up to 15 days.

Non-occluded test

Blanching profiles obtained using the non-occluded test are shown in Figs. 8–10 (note extended ordinates compared to Figs. 1–7). In general, the shapes of the curves obtained in the occluded and non-occluded tests were similar except that Propaderm-C and Topilar (Figs. 9 and 10) showed less distinctive peaks and subsequent falls than in the occluded experiment. However, whereas the occluded profiles for Betnovate and Synalar were very similar (Fig. 1), their non-occluded curves were markedly different (Fig. 8). The results suggest that, under non-occluded testing, fluocinolone acetonide penetrates the skin more slowly from Synalar Cream than does betamethasone valerate from Betnovate Cream, but fluocinolone acetonide is removed more slowly from the site of application. This difference is apparently masked under occlusive conditions, where skin hydration facilitates penetration. Any difference in penetrability of these two steroids is apparently unaffected by the presence of antimicrobial additives (Figs. 8–10).

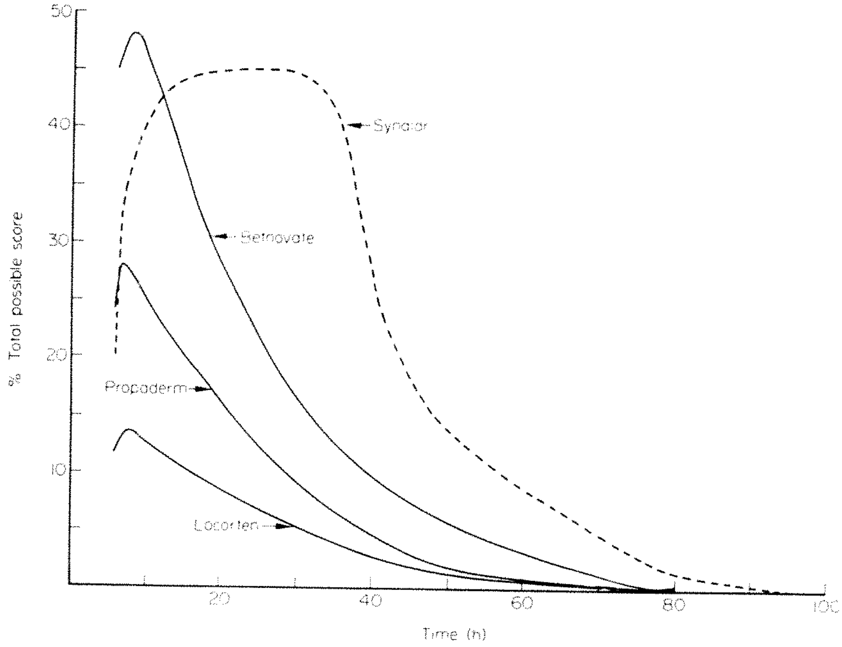

FIGURE 8. Percentage of the total possible score as a function of time for formulations indicated—non-occluded test.

The area under the curve and summed percentage of the total possible score values obtained in the non-occluded test were generally lower than those produced by the occluded procedure (Table 2). When the non-occluded results were expressed as a percentage of the occluded responses the magnitude of the values obtained, especially for Betnovate-C, the Synalar preparations and Topilar, depended

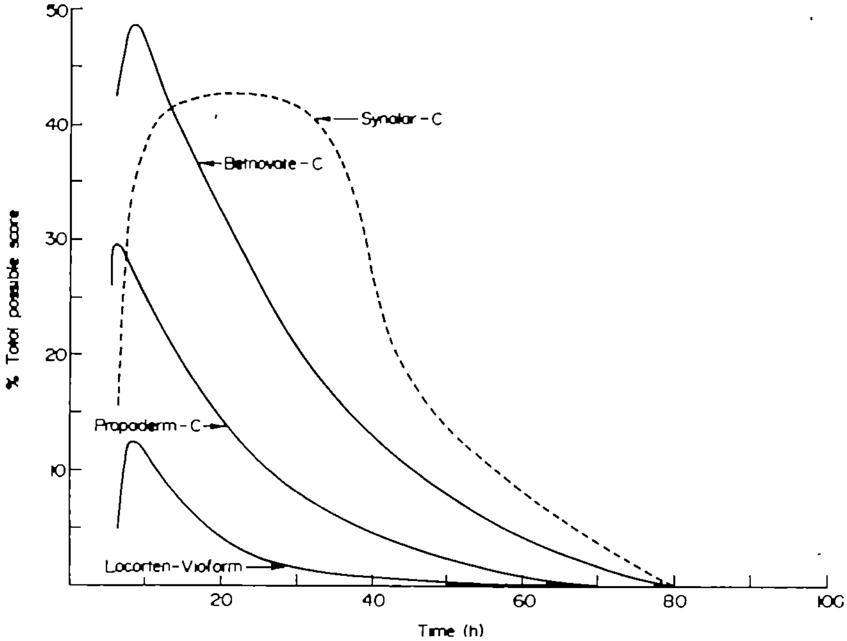

FIGURE 9. Percentage of the total possible score as a function of time for formulations indicated—non-occluded test.

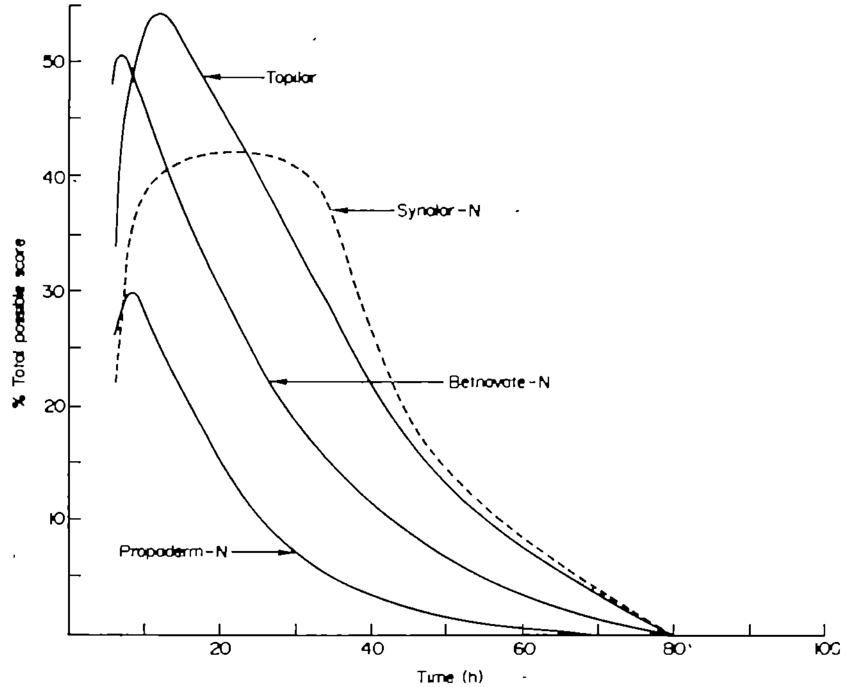

FIGURE 10. Percentage of the total possible score as a function of time for formulations indicated—non-occluded test.

on the parameter (area under the curve or summed percentage of the total possible score) used in the calculation (Table 3).

TABLE 3. Blanching response to selected preparations: non-occluded result expressed as a percentage of occluded value

Preparation	Blanching response	
	Area under the curve	Summed % total possible score
Betnovate Cream	63·5	69·5
Betnovate-C Cream	100	80·0
Betnovate-N Cream	86·9	75.6
Locorten Cream	56·9	56·1
Locorten–Vioform Cream	68·9	68·9
Propaderm Cream	54·6	51·2
Propaderm-C Cream	58·4	52·4
Propaderm-N Cream	54·0	53·9
Synalar Cream	98·9	70·6
Synalar-C Cream	96·1	68·8
Synalar-N Cream	98·8	70·7
Topilar Cream	135	82·2

In the non-occluded test the summed percentage of the total possible scores for Betnovate and Synalar Creams were virtually identical. However, when the results were expressed as the area under the curve for each preparation, the value for Betnovate was only 58% that of Synalar. Calculations showed that, where the shapes of the blanching profiles for steroid formulations were similar (e.g., Propaderm and Betnovate, Fig. 8), the preparations may be arranged in the same rank order by expressing the results either as (a) summed percentage of the total possible scores, or (b) area under the blanching curve. However, where the shapes of the blanching profiles were markedly different (e.g., Synalar and Betnovate, Fig. 8) this was not the case and a truer comparison of the preparations was obtained by comparing area under the curve values.

Re-occlusion of the non-occluded sites 8 days after commencement of the experiment produced very similar results to those of the occluded experiment, the intensity of pallor generally being less in the non-occluded case (Table 2). Pallor also disappeared more quickly and could not be demonstrated on re-occlusion after a further 4 days. Apparently, less steroid reservoir in the skin was produced following non-occluded application.

The basis of an evaluation of corticosteroid preparations by the blanching test assumes that this reflects clinical efficacy. Many manufacturers employ the vasoconstrictor assay in development work and may refer to it in their descriptive literature. It is, therefore, pertinent to consider the likelihood of a skin blanching–clinical efficacy relationship, based on the results reported in this paper.

Dermovate, a new preparation containing a novel steroid, is recommended by the manufacturer as being second to none for treatment of various stubborn inflammatory dermatoses. Logically, therefore, this preparation should score very high in a vasoconstrictor test and in fact it was superior to all others investigated (Table 2). Metosyn, another new formulation, contains the steroid in solution in a gel base designed on physico-chemical principles to maximize its anti-inflammatory effect. Again, this preparation performed well in our vaso-constrictor tests, as did the other gel (Synalar Gel) investigated.

Increasing interest is being shown in the use of potent nonfluorinated steroids (Stewart *et al.*, 1973; Sneddon, 1973). In the present work, Tridesilon, which contains such a steroid, gave a blanching response intermediate to those of Adcortyl and Ledercort, both of which contain twice the percentage of the analogous fluorinated compound. Hydrocortisone and its acetate are recognized as being less potent than newer steroids: Hydrocortisyl and Hydrocortistab were located at the bottom of our classification table of preparations.

The results indirectly suggest that the ranking of proprietary corticosteroid creams and gels based on the skin blanching tests reported in this paper may well reflect their relative clinical efficacy.

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The effect on plasma corticosteroid levels of the short term topical application of clobetasol propionate

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SUMMARY

Thirty-four patients with skin diseases which necessitated treatment with potent topical steroids at a hospital clinic were studied. All the patients received clobetasol propionate ointment (Dermovate) for a period of 2 weeks and sixteen of the patients continued with their treatment for 4 weeks, during which time their plasma corticosteroid levels were measured. Thirty patients had normal levels throughout the whole period of study ($\geqslant 6\ \mu g/100$ ml) and five patients showed a depressed level at some stage during the investigation. Of six patients who were using a large amount of clobetasol ointment (more than 100 g/week) and who had a significant proportion of their body affected by disease (20–80%), two had lowered plasma corticosteroid levels and in four there was no evidence of adrenal suppression.

It has been shown previously that only a very small proportion of those patients being treated with topical steroids have plasma corticosteroid levels below normal (Fry & Wight, 1965; Wilson, Williams & Marsh, 1973; Munro & Clift, 1973). However, those topical steroids which are clinically effective can be absorbed through the skin when applied topically under occlusion to such an extent that depression of adrenocortical function occurs (James, Munro & Feiwel, 1967). Therefore the introduction of a new steroid warrants an investigation of this possibility in patients for whom it is being prescribed.

A new topically active corticosteroid, clobetasol propionate (Dermovate), has been investigated in double-blind comparative trials in several dermatological clinics and has been shown to be highly effective, especially in the treatment of psoriasis (Sparkes & Wilson, 1974).

The following study was designed to determine whether the topical application of clobetasol ointment (0·05%) in typical clinical situations has a detectable effect on plasma corticosteroid levels.

PATIENTS AND METHODS

Adult out-patients who would normally be treated with potent topical steroids were included in the study. However, the following patients were excluded: pregnant women, patients receiving systemic steroids (including oral contraceptive tablets), those who had received systemic steroids within the previous 3 months and those whose treatment or disease was known to affect their hypothalamic-pituitary-adrenal function.

At the first interview the clinical details and diagnosis of each patient were recorded. An estimation was made of the total body area affected by the disease. The patients were carefully questioned about any drugs taken, including their topical steroid regimen. Details of the type of steroid, the amount used per week, the duration of use and whether or not occlusive dressings were employed, were recorded.

At the time of the interview venous blood (10 ml) was collected in a heparinized tube. This was later analysed for plasma corticosteroid levels using a fluorimetric assay (Townsend & James, 1968), and duplicate estimations were carried out on each of the samples. Blood was taken for plasma corticosteroid estimations between 10.00 and 12.30 in the morning or between 1.30 and 4.00 in the afternoon. The time of day at which the samples were taken was recorded and this was consistent throughout the study.

Plasma corticosteroid levels were measured at the beginning, at 2 weeks, and in sixteen of the thirty-four patients, at 4 weeks after commencing treatment with clobetasol. When a patient's lesions were healed after 2 weeks' treatment and no further hospital visit was necessary, the plasma corticosteroid level was not assayed again at 4 weeks. The amount of ointment issued was recorded and at the end of the 2 and 4 week treatment periods all unused tubes were recovered so that the total amount of ointment used could be assessed. No patient received any other topical steroid preparation.

Thirty-five patients (twenty-two male and thirteen female) between the ages of 23 and 67 years were studied. Fourteen of the patients had eczema, seventeen patients had psoriasis and four had lichen planus. One patient was unable to complete even a 2-week period of treatment because of domestic circumstances, the remaining thirty-four patients applied clobetasol ointment twice daily, without occlusion, for either 2 or 4 weeks.

RESULTS

After applying clobetasol ointment for at 2 weeks, one patient's lesions (patient 1) were worse. This patient then discontinued the treatment, although on a later occasion, when clobetasol treatment was recommenced, the lesions improved. Fourteen patients stopped applying the ointment after 2 weeks because their lesions cleared. Of the remaining nineteen patients, eighteen had shown an improvement after 2 weeks, and one patient with lichen planus (patient 13) had experienced no change. After 2 weeks further treatment, seven patients' lesions continued to improve, in seven they were cleared, one patient with eczema showed no further improvement (patient 15) and one patient with pustular psoriasis (patient 12) became worse.

The patients involved in this study were a typical section of those out-patients normally receiving topical steroid therapy in a dermatological clinic. Half the patients (Table 1) had 5% or more of the total body surface affected by their disease (range 5–80%). Consequently, a wide range was seen in the amount of topical steroid applied per week (4–180 g ointment per week).

The mean plasma corticosteroid level was 13.3 μg/100 ml (range 3–25 μg/100 ml) prior to treatment, compared with a mean level of 13·0 μg/100 ml (range 1–24 μg/100 ml) after 2 weeks' treatment with clobetasol ointment. The lower limit of normal adrenal function was taken as ⩾6 μg/100 ml. Thirty patients (86%) had normal plasma corticosteroid levels throughout the whole period of study. Two out of thirty-five patients had an initial plasma corticosteroid level below normal, two out of thirty-four patients and three out of sixteen patients had low plasma corticosteroid levels after 2 and 4 weeks treatment respectively (Table 1).

TABLE 1. The effect of clobetasol propionate ointment on plasma corticosteroid levels

Patient	Area involved (%)	Amount of steroid used (g/week)	Plasma corticosteroid level (μg/100 ml)		
			Initial value	After 2 weeks	After 4 weeks
1 M.B.	<5	37	13*	22*	—
2 H.C.	20	—	17*	—	—
3 A.L.	20	60, 40	8	11	25
4 S.R.	<5	40, 27	12	10	9
5 F.H.	5	43, 42	6	6	9
6 E.C.	<5	20	20*	20	—
7 A.B.	40	93, 90	12*	15*	16*
8 R.W.	<5	15, 11	10	11	9
9 P.L.	20	114	5	8	—
10 R.H.	20	116, 90	3	3	5
11 T.C.	<5	10, 10	13*	17	8
12 E.P.	<5	8, 8	9	10	5
13 A.S.	<5	25	19	27	—
14 K.B.	20	30	8	12	—
15 P.M.	<5	30, 15	12*	9*	11*
16 A.B.	40	103, 120	11	1	10
17 V.O.	<5	30	19	15	—
18 Q.V.	20	30	25	19	—
19 L.M.	5	72, 57	18*	15*	13
20 L.K.	20	30	14	11	—
21 A.G.	<5	4, 4	14*	15	16
22 M.M.	<5	4	22	13	—
23 Y.W.	80	120	12*	12	—
24 G.K.	<5	30	23	24	—
25 C.T.	80	90	11	14	—
26 J.K.	<5	23	11	13	—
27 G.F.	<5	8	14*	14*	—
28 E.C.	5	48	14*	14*	—
29 S.H.	80	150	13	13	—
30 E.P.	<5	23, 15	12*	15*	11*
31 E.C.	5	98, 75	11	9*	10*
32 R.G.	<5	43, 33	13	7	5
33 L.A.	10	180, 137	16	11	10
34 I.B.	5	37	13	10	—
35 B.J.	<5	15	14	16	—

* Blood samples taken in the morning.

DISCUSSION

The results of this study showed that thirty out of thirty-five patients had normal plasma corticosteroid levels at the beginning and end of a period of treatment with clobetasol ointment. Two patients (patients 9 and 10) had a plasma corticosteroid level below normal before beginning treatment with clobetasol. Patient 9, who had an initial level of 5 μg/100 ml, had not applied any topical steroids at all during the week prior to commencing the study. This low level may have been related to the fact that blood sampling was carried out in the afternoon. Two weeks after applying the ointment to approximately 20% of the total body surface (114 g/week) the plasma corticosteroid level was 8 μg/100 ml. Patient 10, who had an initial plasma corticosteroid level of 3 μg/100 ml, had been applying more than 50 g/week of betamethasone valerate ointment with occlusion. This patient, who had 20% of her body surface covered with psoriatic lesions, applied 116 and 90 g/week of clobetasol without occlusion and during this time the plasma corticosteroid levels remained suppressed (3 and 5 μg/100 ml respectively after 2 and 4 weeks treatment.)

Only one patient (patient 16) who began the study with a normal initial plasma corticosteroid level (11 μg/100 ml) had a depressed level (1 μg/100 ml) after 2 weeks treatment with clobetasol. However, although this patient continued to apply the ointment at the same rate (more than 100 g/week) for a further 2 weeks, the plasma corticosteroid level returned to normal (10 μg/100 ml). One possible explanation for this finding is that the healing of the lesions during this period restored the barrier to penetration by the steroid (Scoggins & Kligman, 1965).

Patients 12 and 32, who were diagnosed as having pustular psoriasis and eczema respectively, had less than 5% of their total body surface affected by their disease. Although patient 12 was only applying 8 g/week of clobetasol ointment for 4 weeks, at the end of this time her plasma corticosteroid level had dropped to 5 μg/100 ml. This is an anomaly as this result would not have been expected because of the small amount of body surface affected and the amount of steroid used. Patient 32 applied a larger amount of ointment (43 and 33 g/week) to both feet, the only areas affected, and his plasma corticosteroid level continued to fall (Table 1) throughout the 4-week period of treatment (13, 7 and 5 μg/100 ml respectively).

Whether or not the final plasma corticosteroid levels in these two patients were a result of treatment or merely examples of lower values which might be expected when sampling in the afternoon, would only be established by a more stringent test of pituitary–adrenal function than the one we used. However, it should be noted that four patients (patients 7, 23, 25 and 29) who were using between 90 and 150 g/week with 40–80% skin involvement did not show any depression of plasma corticosteroid levels.

The limitations of random plasma corticosteroid levels as a measure of adrenal function must be appreciated; the more exacting insulin stress test is needed to determine if adrenal responsiveness is suppressed even though a normal plasma corticosteroid level is obtained.

CONCLUSION

Clobetasol propionate is a highly effective topical steroid in the treatment of eczema and psoriasis. The pattern in this study confirms the previous findings (Wilson *et al.*, 1973; Munro & Clift, 1973) that only a very small proportion of patients being treated with potent steroids topically have plasma corticosteroid levels below normal. Short courses of a highly effective treatment, which is then withdrawn until a relapse occurs and which produce only very transient adrenal suppression in a minority of patients would seem preferable to prolonged treatment with a less active steroid. Thus not only would systemic consequences be avoided but the possibility of the more troublesome local side effects would also be lessened.

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Case Report

Erythema multiforme in infectious mononucleosis

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SUMMARY

Infectious mononucleosis may rarely be associated with various patterns of skin eruptions (2–3%). A case is described of a female aged 15 with proven infectious mononucleosis and papular erythema multiforme, unassociated with previous drug therapy. This pattern of reaction can be added to the list of viral infections already known to be associated with erythema multiforme.

CASE REPORT

E.E.W., a 15-year-old girl, presented with a history of malaise for 12 days and a sore throat for 9 days. 5 days prior to being seen she had developed transient puffiness of both periorbital regions followed by bilateral painful swellings of the upper part of the neck. At the same time as the swelling of the eyelids she had noticed development of small, circular, red, raised lesions on the hands, fingers, knees and feet. The lesions were tingling and slightly painful. There was no preceding coryza, cough, headache, vomiting, photophobia or abdominal pain. Her general practitioner had started her on a course of intramuscular penicillin G after the eruption had appeared.

She gave a past medical history of having had occasional sore throats but no herpes simplex. She had had no known contact with glandular fever.

On examination she was a pale rather ill-looking girl with a temperature of 38·2°C. There was a dull red discrete maculo-papular eruption disposed over the dorsal aspects of the hands and finger joints with a few lesions on the palms and palmar surfaces of the fingers. Similar lesions were present on the dorsa of the feet. There was no vesicle formation. On the hard palate there were several petechiae and a well-marked white follicular exudate on both tonsils with no confluent pseudo-membrane formation. There was also a fairly discrete upper cervical and occipital lymphadenopathy but no nodes palpable elsewhere. Examination of the abdomen showed a slightly enlarged spleen but no hepatomegaly.

The clinical appearances were those of a classical papular type of erythema multiforme in association with a follicular tonsillitis, lymphadenopathy and splenomegaly. Together with the history of periorbital swelling (Hoagland's sign) and palatal petechiae, infectious mononucleosis seemed the most likely diagnosis. Follicular tonsillar exudate in infective mononucleosis, however, is unusual, and other conditions, such as a mycoplasma or toxoplasma infection, were also considered.

Investigations

The throat swab showed normal flora on culture and no virus was isolated. The haemoglobin was 13·1 g/100 ml and the white cell count 12,800/mm^3, with a lymphocytosis of 55% and 15% atypical mononuclear cells. The blood film was suggestive of infectious mononucleosis. The monospot screening test was positive. Complement fixation tests, on the fourteenth day of the illness, to *Mycoplasma pneumoniae*, cytomegalovirus, *Coxiella Burnetti*, adenovirus, psittacosis and myxovirus all showed a titre less than 1 in 32. Paul Bunnell Test—a titre of heterophile antibody to sheep erythrocytes at 37°C on day fourteen was of 1 in 640 (unabsorbed), 1 in 320 absorbed guinea-pig kidney, and <1 in 120 absorbed ox erythrocytes.

Progress

On symptomatic therapy the eruption resolved after about 10 days leaving slight residual pigmentation.

DISCUSSION

Erythema multiforme is a distinctive clinical entity which can be precipitated by many and varied agents. In about 50% of cases no known factor is discovered. Herpes simplex and mycoplasma infections are probably the most common precipitating causes. Champion (1972) listed a number of viral infections which have been described with erythema multiforme, including lymphogranuloma inguinale, psittacosis, variola, vaccinia, milker's nodes, orf, mumps and poliomyelitis, but the association of erythema multiforme with infectious mononucleosis is not recorded.

The incidence of skin manifestations in infectious mononucleosis has been reported as occurring in 2–3% of cases (Hoagland, 1960). Hoagland characterized the eruption in thirteen out of 400 patients as being pink and maculo-papular. Sporadic allusion to scarlatiniform, herpetiform and petechial eruptions exist in older literature and there is also the scant reference to urticaria being associated. Apart from these types of eruption (unassociated with previous ampicillin or other drug therapy), definite urticarial lesions have been reported by Alrick & Halprin (1969) and also by Cowdry & Reynolds (1969).

Petrozzi (1971) described a 'palmar dermatitis' consisting of a pruritic erythematous papular eruption occurring only on the palms in a case of infectious mononucleosis.

In summary, the patient reported in this communication showed classical papular erythema multiforme associated with proven infectious mononucleosis and this disease can be added to the already long list of viral infections known to be associated with erythema multiforme.

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History of Dermatology

The history of dermatology in the Sheffield region

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When one looks at Sheffield, today England's fifth city, it is difficult to imagine it as it was at the beginning of the seventeenth century, a small town of about 2200 people huddled round the castle near the confluence of the rivers Don and Sheaf. Before that time the only event of dermatological interest was the establishment of a hospital for poor lepers by William de Lovetot, Lord of Hallamshire, in 1181. Leprosy was then very prevalent and similar institutions were established in many English towns. St Leonard's hospital was built on the land granted by de Lovetot near the bridge over the Don; dissolved in the reign of Henry VIII, nothing now remains but the name of Spital Hill.

Before the eighteenth century there was no doctor in the town, and when one of the manorial servants fell ill in 1639 a doctor had to be brought from Doncaster, bringing his own apothecary with him. The population increased greatly during the eighteenth century and the first medical man to practise there regularly, early in the 1700s, was John Fisher, son of a local minister. By 1750 the population of the whole parish was 20,000 and by 1801 this had grown to 46,000. There were several medical men of note practising in the town at that time, but international fame came to William Buchan (1729–1805), who wrote his Domestic Medicine (1769) while living over an apothecaries' shop in Hartshead. He left Sheffield in 1766 for Edinburgh before the book was first published. It ran to nineteen editions in the author's lifetime and was translated into all European languages, including Russian. He wrote of the connection between lack of cleanliness and skin diseases and for this reason urged cold bathing for everyone, especially for children. He recorded that 'the itch is now by cleanliness banished from every genteel family in Britain. It still, however, prevails among the poorer sort of peasants in Scotland and among the manufacturers in England'. The situation is not much changed today. Buchan died in London and is buried in the cloisters of Westminster Abbey.

During the eighteenth century no fewer than forty-six hospitals, infirmaries and dispensaries were founded throughout the country and when in 1787 an appeal was launched to found one in Sheffield the town was reminded that there was scarcely a city or large town in the kingdom that had not already established its Infirmary. The appeal was successful; the Infirmary was built half a mile from the town on a site which 'can hardly be excelled for the purpose'; a description which the Industrial Revolution was soon to alter. The building was opened in 1797 and half a century later it was recorded that '152,000 sufferers had been cured or relieved, a far greater number than have at any date between 1797 and 1847 been living contemporaries within the compass of Hallamshire.'

In 1829 a group of doctors established a medical school in Surrey Street; the building, bearing the inscription '*Ars longa, Vita brevis*' over its portals, was demolished only in recent years. The Medical School was the oldest of three institutions which combined by charter into a University College in 1897.

In 1832, a year medically noteworthy for a widespread outbreak of cholera which began in Sunderland and ultimately involved a large area of the country including Sheffield, the Public Dispensary was founded. Mr Arthur Jackson, a surgeon of the latter half of the century, wrote: 'It is not easy to realise Sheffield as it was then, with its five churches, one Hospital, no Town Council, no club except a reading room in East Parade, most of the lawyers living at their offices, the manufacturers living close to their works, the doctors living in Fargate, Norfolk Street and Church Street.' By 1854 plans were being made to accommodate inpatients at the Public Dispensary. These beds were opened in 1860 and formed what is now the Royal Hospital.

FIGURE 1. William Dale James (1850–1902).

The first medical man to make a special study of skin diseases in the town was William Dale James (1850–1902), probably led to do so because throughout his life he suffered severely from psoriasis and had only short intervals of freedom. James was a Cornishman, born in Penzance but educated at Wesley College Sheffield and the Sheffield Medical School where he obtained his diploma in 1871. After house appointments at Truro and Hull he settled in practice in Sheffield, and became Surgeon to the Children's Hospital. To further his knowledge of skin disease he visited various clinics, including that of Professor Unna in Hamburg. In 1891 he published 'Notes on a case of lymphangioma cavernosum', in which the histopathology was contributed by Dr Arthur Hall, of whom more later. In writing 'An unusual case of tuberculosis of the skin' (1892), Dr Norman Walker of Edinburgh was co-author. This contact with the dermatological great figures of his time was put to useful purpose, as they sent him photographs and case histories of interesting patients whom they encountered. The collection of six albums of remarkably good photographs is still preserved in the Sheffield University

Library. In 1893 it was decided to form a Skin Department at the Royal Infirmary, and James was the obvious choice as he had by then established a reputation for skill in the treatment of skin disease. He took up the appointment at the end of that year, having been granted three beds and facilities for outpatients to attend at 12 o'clock on Tuesdays and Saturdays! By 1900 he had succeeded in obtaining two bathrooms for dermatological patients and a Finsen light for the outpatient department. His later publications on 'Paraffin acne' (1895) and 'Trade eruptions' (1898) illustrate the problem such eruptions must have been in an industrial town at the end of the nineteenth century. As a man James was admired for his literary ability, amusing in conversation and an excellent storyteller. He also drew witty cartoons with which he embellished the menus of Infirmary dinners; one such menu is still treasured in the Infirmary Medical Staff Room.

For many years James suffered from diabetes and phthisis. For the last year of his life he was an invalid and during that time the House Physician had care of the dermatology patients. Arthur Rupert Hallam was appointed House Physician in April 1902; later in that year Dale James died.

About the same time as James practised at the Infirmary the dermatology at the Royal Hospital was done by a general physician who was one of the most remarkable men to adorn any medical school; Dr (later Sir) Arthur Hall (1866–1951). Arthur was the son of John Hall, a Sheffield surgeon. He was appointed to the staff of the Royal Hospital as physician in 1890 and the following year was performing skin histopathology for Dale James. His publications of dermatological interest about that time were on xerostomia (1898) in which he analysed thirty-nine recorded cases of the disease which was later described by Sjögren in 1933. He also recorded a case of hydroa gestationis (1899). Hall was made Professor of Physiology in 1897, appointed to the Chair of Pathology 2 years later and continued in that appointment until 1906. In 1915 he became Professor of Medicine and retired from that office in 1936. He filled all these appointments with distinction and vacated his earlier chairs only when he found a suitable successor. He continued to practise dermatology as part of his post of physician until 1911, and his interest in skin diseases is still remembered.

At the Infirmary on the death of James one of the physicians, William T. Cocking, took charge of the skin department. He was a native of Sheffield and qualified at University College London, being appointed to the staff of the Royal Infirmary in 1892. He became the first Dean of the Medical School and for many years occupied the Chair of Pharmacology and Therapeutics. During his time most of the skin patients were attended to by the house physician and for several years before his death from pulmonary tuberculosis he was chronically ill and made rare appearances at the Infirmary. He resigned from the staff in 1911 and died a year later.

At this time in 1911 the Royal Hospital appointed E. Fretson Skinner (1880–1944) to take charge of its skin department. His grandfather practised medicine in the town and held the original anatomy license of the medical school which was handed down to son and grandson until a Chair of Anatomy was established in 1897. For a time after his appointment Skinner studied under Sabouraud in Paris. In 1919 he became an assistant general physician. Like most dermatologists at that time he was also venereologist to the hospital and his experience with syphilis increased his interest in neurology which in its turn led him to increasing specialization in psychiatry. In 1931 the Royal Hospital dermatological department was taken over by Rupert Hallam as Skinner gave up dermatology.

Arthur Rupert Hallam (1878–1955) was the eldest son of Dr Arthur Hallam, who was a general practitioner and surgeon to the Royal Infirmary. He studied medicine at Edinburgh University and graduated in 1901 taking the M.D. in 1905. His interest in dermatology was first stimulated when he was appointed House Physician to the Infirmary in 1902 and helped to care for Dale James' patients. After holding posts of House Surgeon and Anaesthetist to the Infirmary, in 1906 he was appointed Medical Officer to the newly created Electrical Department, which over the following decade rapidly grew into a department of diagnostic and therapeutic radiology. In 1911, on the resignation of Dr

Cocking, Hallam was also appointed Medical Officer in charge of the Skin Department. In that appointment he was also responsible for Venereology and in 1914 instituted Evening Venereal Clinics. During the 1914–18 war he served in the R.A.M.C. with the rank of Major. On returning to the Infirmary in 1919 he requested to be allowed to resign the X-ray work while retaining the Dermatology Department, a request which was to be reiterated, until in 1927 Mr J. Wilkie was sent to London to be trained in radiology and took over the work from Hallam. In the same year plans were agreed for a new skin department. In this Hallam incorporated designs in layout which he had seen in clinics abroad visited during his holidays. The size of the department was to be enlarged in 1949 when it was named after Hallam. It moved to the Hallamshire Hospital in 1969 to an entirely new and purpose built clinic which Hallam would find not entirely unfamiliar, as many of his designs are still incorporated.

FIGURE 2. Arthur Rupert Hallam (1875–1955).

By 1930 he had a full time clinical assistant, and when Skinner gave up dermatology, Hallam was the only consultant dermatologist in an area which stretched from the Pennine chain to the coast.

An original member of the British Association of Dermatology, he was President in 1936 when the Association met in Sheffield, and was later elected an Honorary Member. He helped found the North of England Dermatological Society and was its President in 1930, the year after its foundation.

One of his interests was papular urticaria (1927, 1932). He was aware that Hutchinson thought that the bites of insects were the cause, and his investigations into the effects of admitting children to hospital with their mattresses from home brought him near to the modern acceptance of Hutchinson's theory. Another interesting experiment was his establishment of open air centres around Sheffield and in the Infirmary grounds for the daily attendance of sufferers from lupus vulgaris (1943). In one good summer he was encouraged by the improvement even in those in the smoke laden air round the Infirmary. The following wet summer dampened his and the patients' enthusiasm.

His other publications were on erythema multiforme (1933), the prognosis of psoriasis (1934) and miliary lymphocytoma (1939), the last being written with H.R.Vickers, who became his colleague and successor.

Hallam obtained the M.R.C.P. in 1936 and was elected to the Fellowship in 1940. He retired in 1944, settling in the West Country where he pursued his hobbies of painting, gardening and walking. His skill at drawing is illustrated in the self-portrait reproduced here.

LEICESTER

Leicester, in the Southernmost part of the Sheffield Region, is the only other area where dermatology was established as specialist practice before the turn of the century.

In 1898, Dr Alec P. Bremner founded the Leicester Institute for Diseases of the Skin in a building in Church Gate in the town centre. The original building has now been replaced by a shop. Bremner was a Glasgow graduate (L.R.C.P., L.R.C.S. 1876) and was awarded the Chesterfield medal in Dermatology at St John's Hospital, London, in 1896. He was a fellow of the London Dermatological Society and became its President in 1918–19. The society became the St John's Society in 1926 when Bremner was made Vice-President. He died in 1926. He had been succeeded at the Institute in 1925 by Dr F.A.E.Silcock (1892–1955), a Belfast graduate who qualified M.B., B.Chir. in 1915. After house appointments he became R.M.O. at the Belfast Fever Hospital and in 1923 was appointed Medical Superintendent to the Isolation Hospital in Leicester. After obtaining the M.D. in 1925 he concentrated on dermatology, working at the Institute until in 1934 he was appointed also to the honorary staff at Leicester Royal Infirmary. The following year the Institute merged with the Infirmary. Silcock was particularly interested in occupational dermatitis and during the Second World War was a member of the Ministry of Labour's panel for industrial dermatitis. At that time he was a frequent contributor to discussions on this subject at the Royal Society of Medicine. He was also one of the first British dermatologists to investigate the properties and value of water miscible bases. In 1950 failing vision forced him to retire from the Infirmary and he became completely blind before his death in 1955.

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Comment

THE PITUITARY MELANOTROPHIC HORMONES

Whether or not a pituitary melanotrophic hormone plays a role in the physiology of normal human melanin pigmentation is not clear. Hypophysectomy does not cause the negro skin to lighten (Fitzpatrick & Mihm, 1971), and only the administration of large amounts of the anterior pituitary hormone, adrenocorticotrophin (ACTH), can increase pigmentation (Lerner & McGuire, 1964). However, ACTH has only approximately 1% of the melanotrophic activity of the two major melanocyte stimulating hormones, α- and β-MSH (Li, 1959). Large doses of either of these hormones will increase pigmentation by increasing both the number of melanosomes in the keratinocytes as well as the rate of melanogenesis in the melanocytes (Snell, 1967). The structure of α-MSH is identical in all species examined, and is the same as the first thirteen aminoacids of ACTH, with an acetylated N-terminus and amidated C-terminus. α-MSH is secreted by the pars intermedia of the pituitary (Atwell, 1919; Dubois, 1972) and was originally called intermedin. The human pituitary gland, however, does not have a distinct intermediate lobe (Romeis, 1940), which could explain the repeated failure to identify α-MSH in human pituitaries (Lerner, Upton & Lande, 1968; Scott, Lowry & Ratcliffe, 1973). Using a radio-immunoassay for α-MSH, Abe and his colleagues (1967a) were only able to account for 2% of the melanotrophic activity of human pituitary extracts; it therefore seems likely that an intermediate lobe may be essential for the formation of α-MSH (Scott & Lowry, 1974).

The structure of the other human melanotrophic hormone, β-MSH, was first described by Harris (1959). Unlike the eighteen amino acid β-MSH hormones of most species, human β-MSH has an extra four amino acids at the N-terminus. It is structurally related to larger peptides called lipotrophins which have been isolated from ox, sheep (Birk & Li, 1964) and human pituitaries (Cseh *et al.*, 1972). The two lipotrophins isolated (β- and γ-LPH) possess ninety-one and fifty-eight amino acids respectively, and both peptides contain the entire amino acid sequence of β-MSH. The biological effects of the lipotrophins include melanotrophic properties in amphibians and lipolytic properties in rabbits but only when they are present in pharmacological concentrations (Lohmar & Li, 1968). They also have sebotrophic activity in rats (Thody & Shuster, 1971) and can decrease plasma calcium in rabbits (Chrétien, Lis & Gilardeau, 1973). Recent chromatographic evidence strongly suggests that β-MSH is not present either in human pituitaries (Scott & Lowry, 1974) or plasma (Bloomfield *et al.*, 1974) and that the main β-MSH-like immunoreactive material found in pituitary and plasma resembles the larger molecule, β-LPH. In some β-MSH radio-immunoassays purified human β- and γ-LPH cross-react completely (Gilkes *et al.*, 1974). The important implication of this observation is that such systems may well have been measuring β- or γ-LPH, and not the smaller molecule, which we know as human β-MSH.

The levels of β-MSH measured by radio-immunoassay have been shown to be raised in the same situations as ACTH, namely Addison's disease, Nelson's syndrome and ectopic ACTH syndrome (Abe *et al.*, 1967b; Thody & Plummer, 1973). The diurnal variation of β-MSH is lost in patients with Cushing's disease (Abe *et al.*, 1969) and the levels of β-MSH and ACTH correlate very closely in human foetal pituitaries (Kastin *et al.*, 1968). In patients with skin pigmentation, not associated with pituitary or adrenal abnormalities, β-MSH levels are normal (Abe *et al.*, 1969). Although increased levels of MSH have been found in the urine and blood of pregnant women after the second month of gestation (Shizume & Lerner, 1954), the increased skin pigmentation sometimes seen in pregnancy and in the latter days of the menstrual cycle (Snell & Turner, 1966) could be attributed to the melanotrophic activity of oestrogens and progesterone (Snell & Bischitz, 1960). Recent interest has been focussed on the role of β-MSH in Parkinson's disease. Increased immunoreactive β-MSH levels have been found in patients with bilateral Parkinsonism (Shuster *et al.*, 1973) and injections of synthetic human β-MSH aggravated the associated tremor (Cotzias, Van Woert & Schiffer, 1967). However, at this time the exact mechanism by which these changes occur still remains speculative.

Several of the hormones of the anterior pituitary have been shown to be under the control of hypothalamic hormones which exert either release or inhibitor control over anterior pituitary secretion. There is now good evidence that the hypothalamus inhibits the release of MSH in some mammals and that this is effected by a hypothalamic hormone (Kastin, 1965). When administered to rats this substance, designated MSH-release inhibiting factor (MIF), increased the pituitary MSH and decreased the amount of MSH released into the blood (Kastin *et al.*, 1969). A similar effect was shown in rats with a human hypothalamic extract (Kastin & Schally, 1967). Interestingly, the structure of bovine MIF (Pro–Leu–Gly–NH_2) is identical to the side chain of oxytocin (Nair, Kastin & Schally, 1971). Although synthetic MIF injected into man does not lower levels of MSH in patients with Nelson's syndrome or Addison's disease (Kastin, Gual & Schally, 1972), some improvement has been noted in the rigidity and tremor of patients with Parkinsonism (Kastin & Barbeau, 1972).

To date, a well-defined function for the melanotrophic hormones in the physiological control of human pigmentation has not been found. Unfortunately, most investigators in this field have used the synthetic peptides α- and β-MSH in their studies, neither of which may occur naturally in man. Adrenocorticotrophin and the lipotrophins cause increased skin pigmentation only when present in greatly increased concentrations, and under normal conditions would seem to play no part in the regulation of skin pigmentation.

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British Journal of Dermatology (1974) **91**, 357.

Correspondence

CIVATTE BODIES AND APOPTOSIS

SIR, The ultrastructure of Civatte bodies in lichen planus, as illustrated in the recent paper by El-Labban & Kramer (1974), is typical of 'apoptosis', the morphologically distinct type of cellular death described by Kerr, Wyllie & Currie (1972). This process has been reported under a variety of names: Councilman bodies in the liver (Klion & Schaffner, 1966); tingible bodies in the germinal centres of lymph nodes (Swartzendruber & Congdon, 1963); dyskeratotic cells in the skin after ultra-violet radiation damage (Wilgram *et al.*, 1970); and karyolytic bodies in the epithelium of duodenal crypts after whole body irradiation of mice (Hugon & Borgers, 1966).

Fig. 2 of El-Labban & Kramer's article (1974) shows well the earliest stages of apoptosis with condensation of the nucleus and cytoplasm and surface budding to produce membrane-bound particles. A later stage of this process is illustrated in their Fig. 3, and this electron micrograph is similar to Fig. 5 in Kerr & Searle's paper (1972) which shows apoptosis in an epithelial tumour of the skin. Furthermore, the dense nuclear fragments seen in apoptosis may be confused ultrastructurally with sections through the chromosomes in cells undergoing mitosis.

It is important to distinguish apoptosis from other forms of cell death, since it has been shown to occur under normal as well as pathological conditions, and there is much evidence that it plays a complementary but opposite role to mitosis in regulating the size of cell populations. Its prominence in lichen planus, where a disturbance in cell turnover rates occurs, is thus of interest.

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Book Reviews

Parents' Guide to Allergy in Children. CLAUDE A. FRAZIER (1973) New York: Doubleday & Company Incorporated. Pp. 338. Price £3.25.

This book is written by an American allergist well known both as an author and for his investigations, for example of insect sting allergies. There is much in it to interest any doctor concerned in treating children, and many places where he must wonder how often he has missed a particular cause when he has been content to make such a diagnosis as atopic dermatitis, urticaria, or even stiff-neck or bed-wetting.

The general impression from the book is that by exhaustive attention to history-taking, and manipulation of the diet and the environment, a wide range of major and minor ills could be dispelled. This is certainly true, but how often?

The book is written for the American parent, and lists of pollens and other hazards are inevitably not comparable in other countries. Although, no doubt, it is only by having informed parents that many genuine allergic disorders can be unravelled, it is doubtful whether many parents in this country will find that this relatively large book helps them to put their children's illnesses in perspective, or whether it will greatly improve their relationships with their doctor! The book is, however, salutary reading for the doctor.

R.H.CHAMPION

Sexually Transmitted Diseases. LESLIE NICHOLAS (Ed.) (1973) Springfield: Thomas. Pp. xiv+244, illustrated. Price £5.90.

Sexually Transmitted Diseases. C.B.S.SCHOFIELD (1972) Edinburgh and London: Churchill Livingstone. Pp. 248. Price £1.25.

For historical and administrative reasons few dermatologists in Britain now treat venereal diseases, although they do so in most other countries. The approved training programme in dermatology in Britain now rightly includes experience in a clinic for venereal diseases, but many men who trained between 1950 and 1970 enjoyed no such experience and for them in particular these two very different books on 'Sexually Transmitted Diseases' are of considerable importance. Gonorrhoea has become in many parts of the world the commonest bacterial infection of adults and the genital infection is asymptomatic in some 80% of females and 20% of males; extragenital lesions are therefore becoming increasingly common, since treatment is often not sought in the early stages of the infection. The techniques for the serological diagnosis of syphilis have become more refined and their significance is better understood. The dermatologist who still writes on the pathology form, 'WR please' needs to read these books. A variety of factors have combined to intensify, and diversify, the depredations of the genital strains of Herpesvirus hominis, and the dermatologist must be well informed on all aspects of its natural history, for such infections frequently come his way.

The first of these books, edited by Dr Nicholas, brings together a collection of papers read at meetings of the American Academy of Dermatology, and a paper by Dr R.R.Wilcox on 'The Treponemal Evolution', which he presented at a Symposium at St John's Hospital, London, in 1971. Of particular interest to the dermatologist who does not regularly treat venereal disease, are papers by Stephen J.Kraus and by Denny L.Tuffanelli on false positive reactions, by Harry Pariser on asymptomatic gonorrhoea, by Charles J.McDonald on extragenital gonococcal infections and by André Nahmias and his colleagues on herpes simplex virus infections.

The second book, by Dr Schofield, of Glasgow, is intended for medical students. It provides a systematic clearly written account of the common and the rarer sexually transmitted diseases. Each chapter ends with a useful list of selected references for further reading. This book can be strongly recommended not only to the medical student, but also to those dermatologists whose venereology has not advanced beyond the Wassermann era, as a convenient and inexpensive text.

ARTHUR ROOK

British Journal of Dermatology (1974) **91**, 359.

Review

· Cutaneous reactions to food and drug additives

Many substances are now incorporated into foods, beverages and drugs as additives for purposes of colouring, flavouring, preserving, thickening, emulsifying and stabilizing. Some of these chemicals are identical with or cross-react with drugs. During the course of a year every person in this country consumes on average some 3 lb of chemical substances which are not normal constituents of food, and the quantity is increasing (*Lancet*, 1969). The total number of known food additives exceeds 20,000. The majority of these are present in very small quantities but some are known to be poisonous to animals in such amounts. Food additives are controlled by law in most countries although thousands have not been subjected to official testing for adverse reactions. This raises fears of danger to the health of this and subsequent generations. Thus the potential hazard of these chemical additives must be regarded with particular concern because of extensive and prolonged use of these agents in the diet.

It has been established that hidden allergens in the form of additives cause a wide variety of hypersensitivity reactions. Drug reactions comprise an important proportion of medical practice and in many cases the physician who prescribes the drug recognizes the significance of any reaction. In addition to this, individual intolerance and idiosyncratic reactions may occur to chemical agents present in the prescribed drug, besides the main ingredients (Lockey, 1971). These reactions may inadvertently be attributed to the main drug.

Despite of this very extensive use of additives the incidence of reactions is unknown, many being undiagnosed. In this review a few well documented cutaneous reactions to selected additives are described and potential adverse effects are discussed.

FLAVOURING AGENTS

These are mostly mixtures of esters such as ethyl butyrate and amyl acetate. Cyclamate (sodium cyclohexyl sulphamate) has, until recently, been used extensively as a sweetening agent, and quinine is employed as a bitter. Flavour potentiators, such as monosodium glutamate, enhance the taste of other substances without themselves imparting any flavour of their own.

Cyclamate

Despite the massive amounts of cyclamates consumed, reported cutaneous reactions are very uncommon. The only well documented reaction is of photodermatitis (Kabori & Araki, 1966; Lamberg, 1967). The mechanism is thought to be one of photo-allergy rather than phototoxicity.

Quinine

Quinine is present in numerous 'over-the-counter' cold and headache remedies. It is present in 'bitter-lemon' and 'tonic water', which may contain as much as 5 mg/100 ml. Cinchonism, which consists of nausea, vomiting, disturbance of vision, tinnitus and nerve deafness, may occur from an overdose of quinine. Such a symptom-complex may also result when small quantities of the drug are taken as in 'tonic-water'. In this case, the patient is said to have an idiosyncrasy to quinine. One of the most common eruptions caused by quinine is a generalized scarlatiniform rash (Calnan & Caron, 1961). Purpuric eruptions have been well documented and may, or may not be thrombocytopenic. The mechanism of quinine-induced thrombocytopenic purpura is identical to that due to quinidine or Sedormid. The drug acts as a hapten combining with the platelets to provide an antigenic stimulus. The antibody produced in response to this stimulus combines with the platelet–drug complex causing platelet agglutination. In the presence of complement, platelet lysis may also occur. Belkin (1967) described in detail a patient who developed such purpura due to hypersensitivity to the quinine contained in tonic-water. Topical sensitization may take place from hair tonics and other hair preparations containing quinine. The ingestion of quinine may produce eczematous contact-type dermatitis in sensitized individuals (Fisher, 1966.) Other less common reactions to quinine include bullous and fixed eruptions (Derbes, 1964) and lichenoid eruptions (Almeyda & Levantine, 1971).

Cinnamon oil

Cinnamon oil is used for flavouring foods, toothpaste, chewing gum, Vermouth, various other aperitifs and bitters, and beverages of the cola-type. Contact dermatitis due to oil of cinnamon may occur in bakers, sweet manufacturers, cooks, and from the use of certain toothpastes (Fisher, 1966). Ingestion of cinnamon oil may produce an eczematous contact-type flare-up in individuals previously sensitized by contact with the oil (Leifer, 1951). As it cross-reacts with balsam of Peru ingestion of this substance may produce a contact dermatitis in a patient sensitized to balsam of Peru (Hjorth, 1961).

Vanilla

Vanilla cross sensitizes with balsam of Peru, benzoic acid, orange peel, cinnamon and clove. Whether or not an eczematous contact-type dermatitis results from systemic sensitization is not known (Mitchell, 1971).

Salicylates

These may be used to flavour ice cream, chewing gum, soft drinks and cake mixes (Lockey, 1971). Ingestion of the foods by aspirin-sensitive patients may result in such phenomena as urticaria.

EMULSIFIERS

These are substances that aid the uniform dispersion of oil in water. They form emulsions such as margarine, ice cream and salad cream. In 1958 in Western Germany and 1960 in the Netherlands, there were epidemics of the so-called 'Dutch Margarine disease'. The disease was attributed to an identical emulsifier used in both countries for the preparation of certain brands of margarine. The eruption had a sudden onset, becoming generalized in 2–24 h, and was accompanied by severe pruritus. Prodromal symptoms were slight. The rash started on the face and hands and spread to the trunk and legs. The individual lesions consisted of small pink to bright red macules which soon developed an urticarial element. These would fade away in a few days or, in severe cases, develop into purpuric lesions or become larger in size. Some patients had high fever, felt ill and developed conjunctivitis, sore throat and swollen lips. Mali and Malten (1966) reviewed the Dutch epidemic in detail and likened the condition to erythema multiforme in order to emphasize that opinions were divided as to the exact aetiological significance of the margarine.

COLOURING AGENTS

These are added to foods to make them more visually attractive. They fall into three groups; (1)

natural pigments derived mainly from plant materials; (2) inorganic pigments including metals; and (3) synthetic coal tar derivatives.

The list of permitted colours varies so much from country to country that there is not a single dye which is permitted in every country.

Coal tar dyes are complex, low molecular weight synthetic substances. A number of them have been banned because of toxicity and carcinogenicity, the most notorious being 'butter yellow', which was banned in 1919.

Red no. 2 dye (amaranth) is the most widely used and highly suspect of the coal tar dyes. It is used to produce the cherry-red hue of soft drinks and is added to many other food products.

Tartrazine

This is a pyrazole derivative, an aniline dye yellow in colour, which includes heterocyclic rings and acidic groups. It is present in a great variety of products, including dry drink powders, carbonated drinks such as 'Tang' and coloured sweets and as a dye to colour certain proprietary drug capsules, e.g. dexamethasone, and in toothpastes and many cosmetics.

Lockey (1971) first described asthma and urticaria caused by tartrazine. Since then many instances of similar reactions have been reported.

Crisp (1971) recorded non-thrombocytopenic vascular purpura due to tartrazine sensitivity in a 22-year-old woman. Routine examination and investigations for purpura were negative. After keeping a food diary the subject discovered that eating butter or margarine was invariably followed by bleeding and this was confirmed by provocative tests. Scratch patch tests with tartrazine dye in a 1:1000, and later 1:100 dilution produced erythema, induration, vesiculation and petechiae 24 h later. More recently, Michaëlsson, Petterson & Juhlin (1974) have provided strong evidence that food dyes, including tartrazine, can play a part in Henoch–Schönlein purpura.

Phenolphthalein

Phenolphthalein, a triphenylmethane dye, is present in many proprietary laxatives and also in proprietary analgesic preparations such as Hypon and can cause a fixed drug eruption. Related substances are used as food dyes.

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British Journal of Dermatology (1974) **91**, 363.

News and Notices

Announcement

The European Society for Dermatological Research and the Society for Investigative Dermatology will hold their second joint meeting in the RAI Congrescentrum in Amsterdam on 9–13 June 1975.

Attendance at the scientific sessions will be open to all members, contributors and guests.

Non-members who wish to present a paper are kindly requested to submit an abstract. They will be invited to attend the meeting as either contributors or guests.

Information about the meeting can be obtained from Ch.M.Lapiere, ESDR Secretary, Department of Dermatology, Hôpital de Bavière, B–4000 Liege, Belgium (*tel.* 041–43.93.72).

Corrigenda

In the paper by W.J.Cunliffe, R.A.Forster & M.Williams 'A surface microscope for clinical and laboratory use' which appeared in the June issue of the Journal (Vol. **90**, p.619) the legends for the colour plate facing p.620 should have read as follows:

PLATE 1. Area of back showing pilo-sebaceous duct orifices and comedones (×20).

PLATE 2. Eczema (×20).

PLATE 3. Psoriasis (×20).

PLATE 4. Purpura (×20).

Uneasy lies the head
that suffers from
seborrhoeic dermatitis.
Uneasy lies the hair
when the scalp is treated
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M.K. Polano (Leiden), and *R. Schuppli* (Basel)

Index Vol. 148, No. 4, 1974

Vogel, A. und Narasimhan, S. (Zürich): Struktur und Schicksal der Desmosomen beim Morbus Darier
(Structure and Fate of Desmosomes in Darier's Disease)

Jung, E.G. und Bohnert, E. (Heidelberg): Chronisch polymorphe Lichtermatose. Untersuchungen an Lymphozyten *in vitro*
(Chronic Polymorphic Light Eruption. *In vitro* Lymphocyte Studies)

Stevens, D.A. (Stanford, Calif.): Collection for Study of Small Amounts of Vesicular Fluid from Vesiculobullous Skin Diseases

Sehgal, V.N. (Panaji): Significance of the Local Sweat Response in the Diagnosis of Leprosy

Fusaro, R.M. and Johnson, J.A. (Omaha, Nebr.): Photoprotection of Patients Sensitive to Short and/or Long Ultraviolet Light with Dihydroxyacetone/Naphthoquinone

Dulanto, F. de; Armijo-Moreno, M., and Camacho-Martínez, F. (Granada): Tuberculoid Granulomata in Regional Lymph Nodes of Malignant Cutaneous Melanoma

Carapeto, F.J. and García-Pérez, A. (Salamanca): Acantholytic Keratosis

Zergollern, Ljiljana; Laktić, Nikda; Schmutzer, Ljerka, and Puretić, Štefanija (Zagreb): Focal Dermal Hypoplasia

Parish, L.C. and Polin, J.I. (Philadelphia, Pa.): Bromoderma in Pregnancy

Gutzwiller, P. (Basel): Zum Problem der Kortikosteroid-Allergie
(The Problem of Corticosteroid Allergy)

The Interesting Case · Der interessante Fall · Un cas intéressant

Vončina, D. (Ljubljana): Osteoma Cutis

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs.', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

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[illegible]ill be submitted without the original typescript to the author for proof-correction (see under [illegible]d should be returned to the Editor within three days. Major alterations from the text cannot

British Journal of Dermatology, Volume 91, Number 3, September 1974

CONTENTS

Clinical and Laboratory Investigations

PAGE

The Premature Ageing Syndrome 243
J. J. H. GILKES, D. E. SHARVILL AND R. S. WELLS

The Papulonecrotic Tuberculide 263
J. G. L. MORRISON AND E. D. FOURIE

Deoxyribonucleic Acid (DNA) in Cattle Skin Washings 271
R. M. MABON

Monolayer Culture of Cells from Psoriatic Lesions 275
J. R. COOPER AND M. A. COWAN

Immunoglobulin and Complement Deposits in the Skin in Inflammatory Facial Dermatoses—An Immunofluorescence Study 281
E. ABELL, M. M. BLACK AND R. MARKS

Elastosis in Chronic Radiodermatitis—An Ultrastructural Study 287
M. LEDOUX-CORBUSIER AND G. ACHTEN

Black Grain Mycetoma—A Study of the Chemistry Formation and Significance of the Tissue Grain in *Madurella mycetomi* Infection 297
G. H. FINDLAY AND H. F. VISMER

Palmer and Finger Varicosities of the Aged 305
A. N. G. CLARK, D. H. MELCHER AND PATRICK HALL-SMITH

Elution of Antibodies from the Lymphocyte Membrane in Certain Dermatoses 315
R. H. CORMANE, F. HAMERLINCK AND S. HUSZ

Pharmacology and Treatment

Tetracycline for the Treatment of Pityriasis Lichenoides 319
THADA PIAMPHONGSANT

Comparative Bio-availability of Proprietary Topical Corticosteroid Preparations; Vasoconstrictor Assays on Thirty Creams and Gels 323
B. W. BARRY AND R. WOODFORD

The Effect on Plasma Corticosteroid Levels of the Short Term Topical Application of Cobetasol Propionate 339
STUART R. WALKER, LYN WILSON, LIONEL FRY AND V. H. T. JAMES

Case Report

Erythema Multiforme in Infectious Mononucleosis 345
DONALD M. WILLIAMSON

History of Dermatology

The History of Dermatology in the Sheffield Region 347
RONALD CHURCH

Comment

The Pituitary Melanotrophic Hormones ...... [illegible]
J. J. H. GILKES

Correspondence

Civatte Bodies and Apoptosis ...... [illegible]
DAVID WEEDON

Book Reviews ...... [illegible]

Review

Cutaneious Reactions to Food and Drug Additives ...... [illegible]
ASHLEY LAVENTINE AND JOHN ALMEYDA

News and Notices ...... [illegible]

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4.5.75

Volume 91, Number 4, October 1974

British Journal of Dermatology

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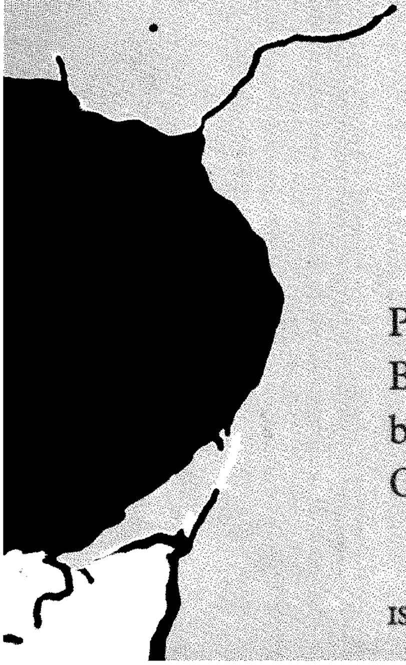

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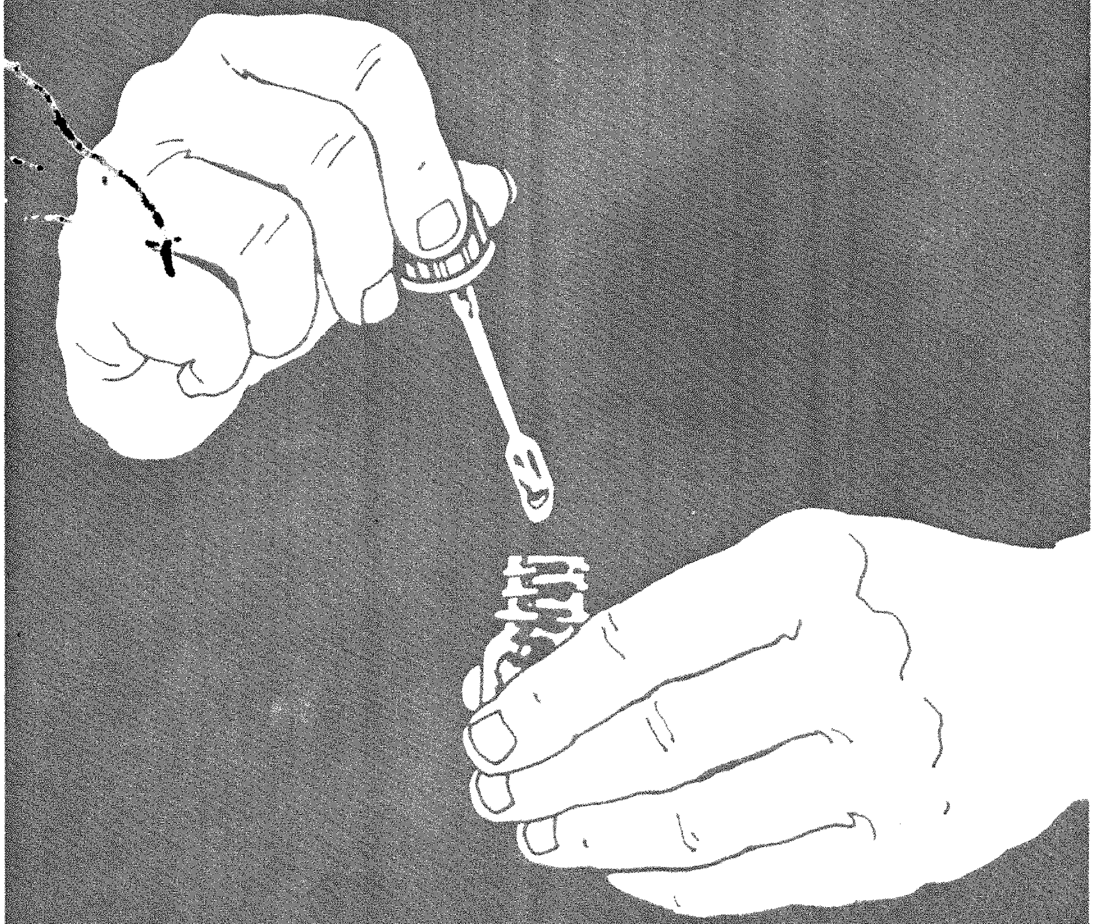

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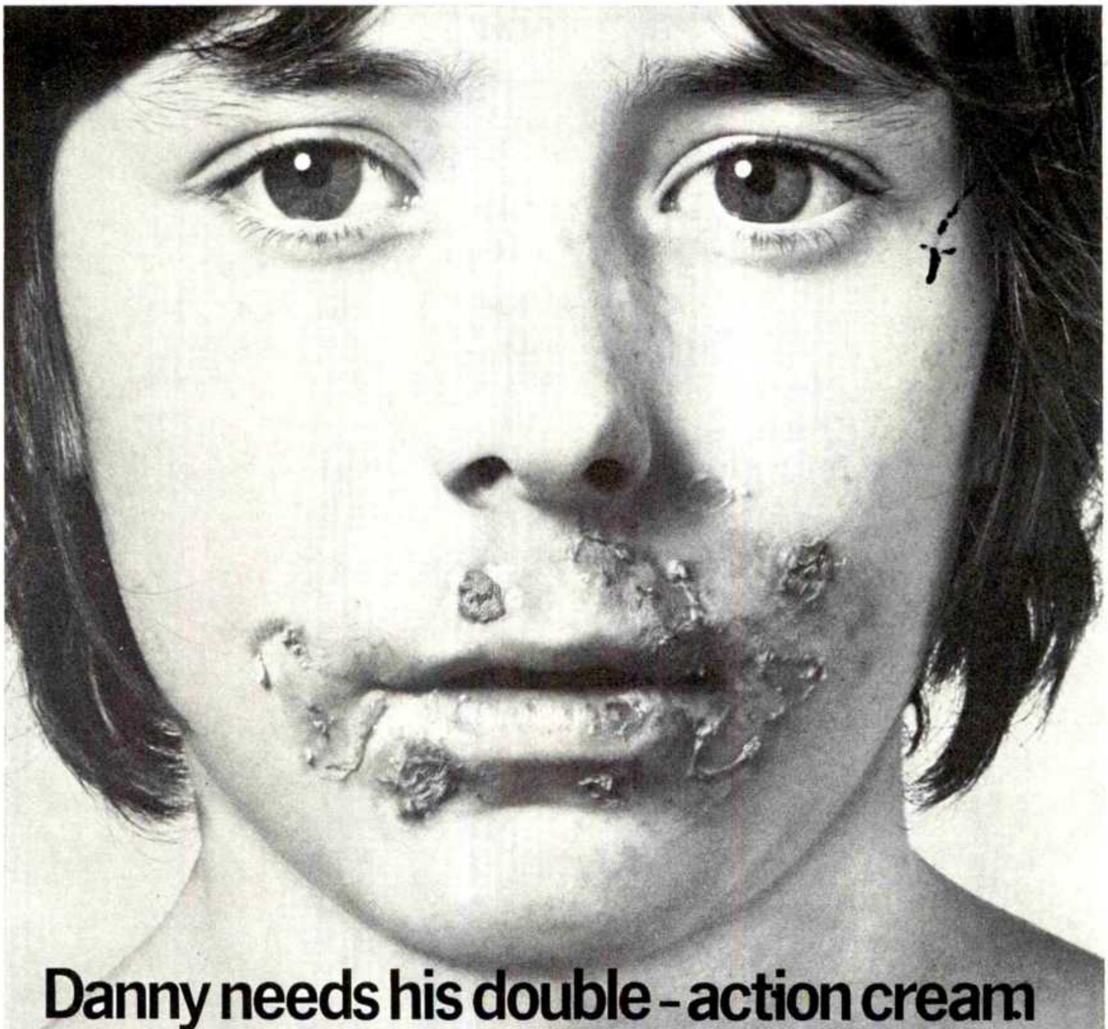

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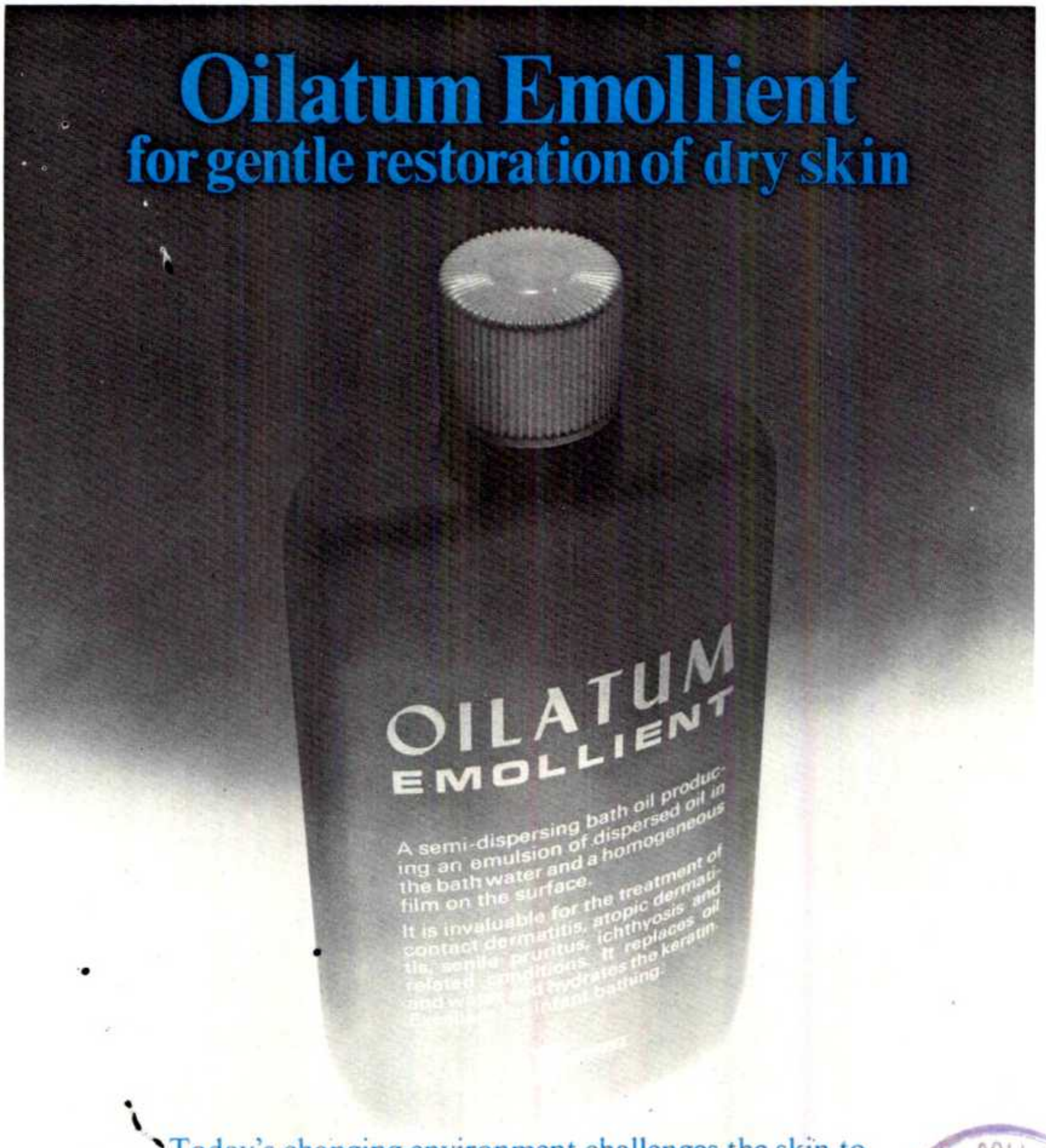
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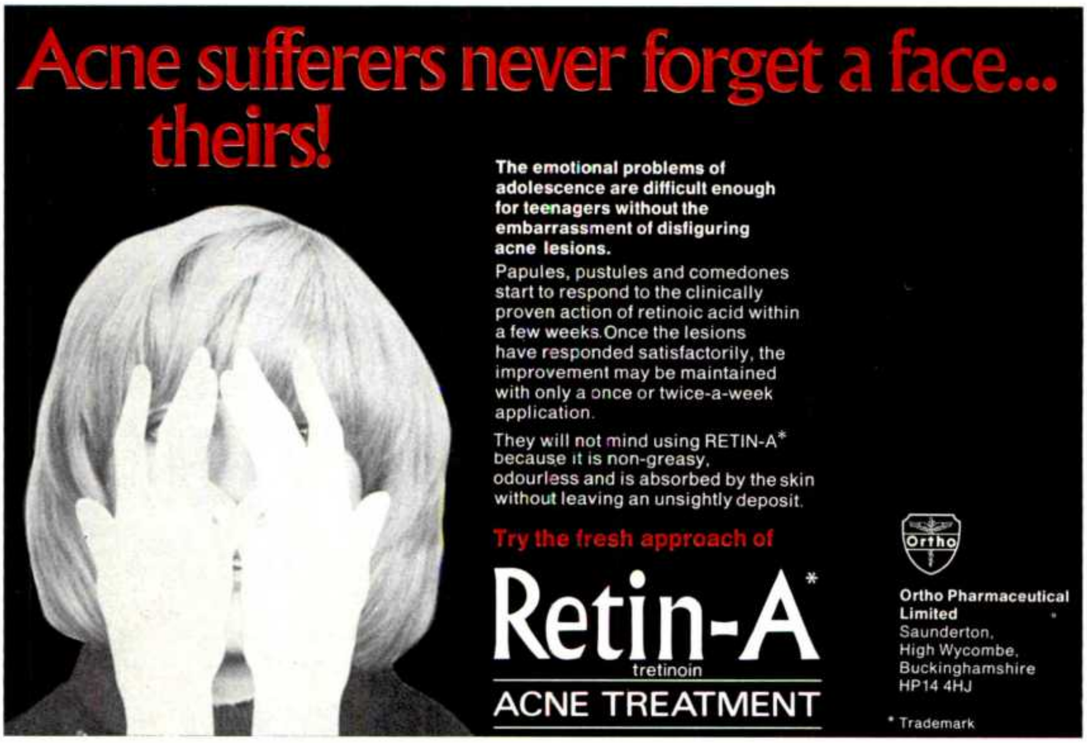
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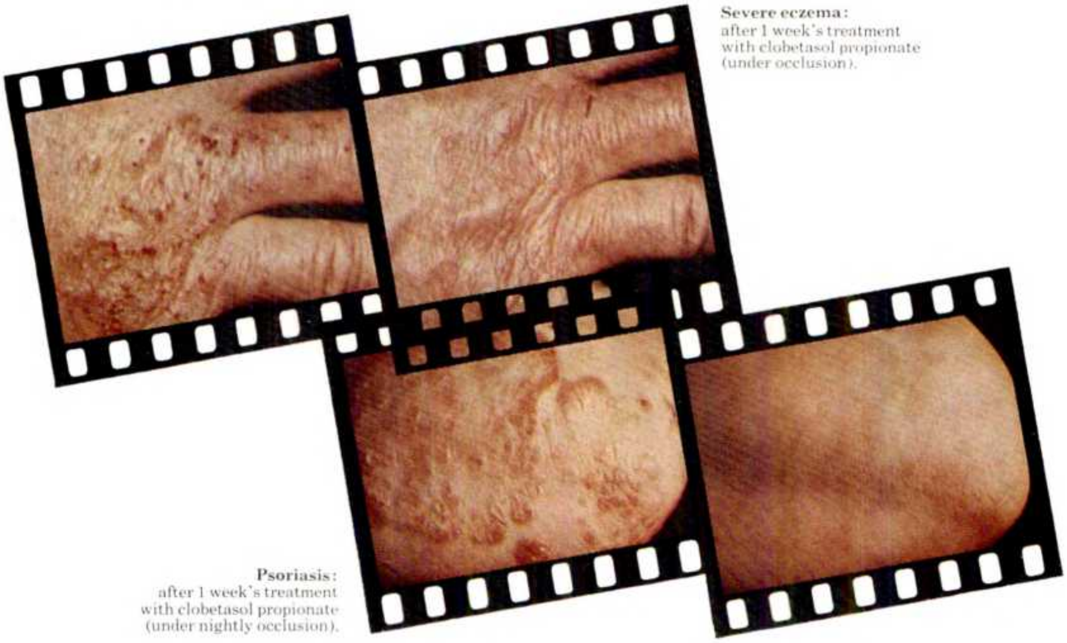

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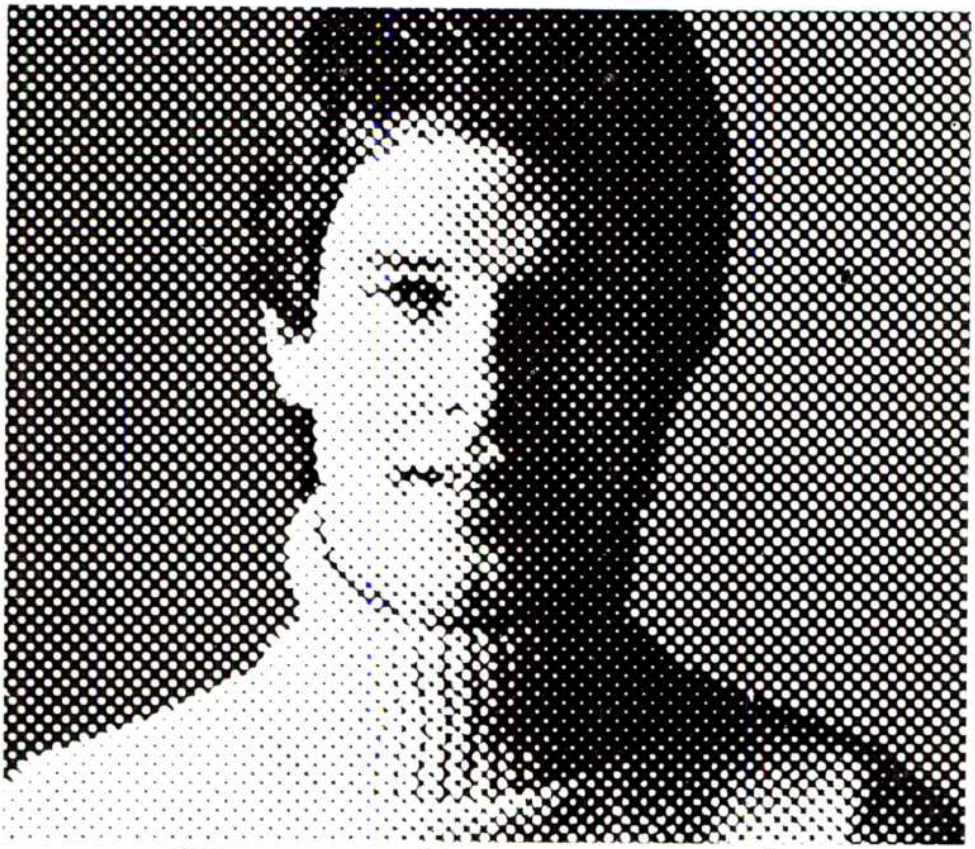

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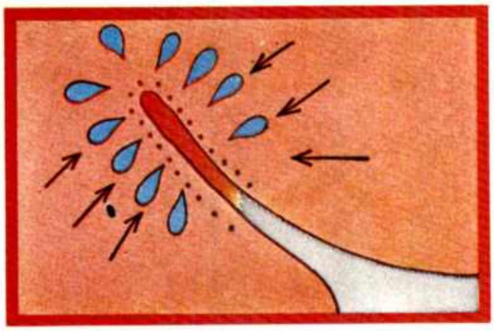

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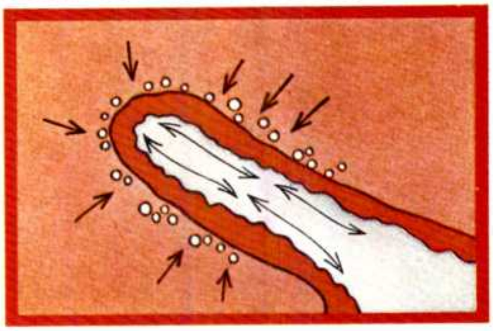

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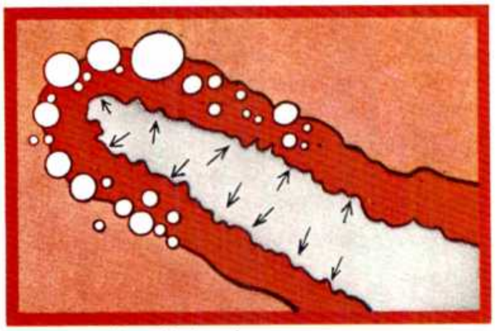

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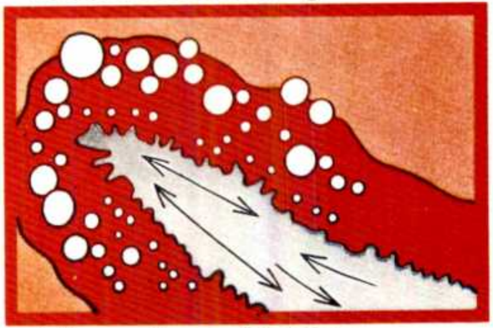

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Index Vol. 148, No. 6, 1974

Weirich, E.G. und Longauer, J. (Basel): Stearadenotroper Effekt von Androgenen am Kaninchenohr. Histometrische Untersuchungen zur Talgdrüsenaktivität. 1. Mitteilung (Stearadenotrophic Effect of Androgens in Rabbit Ear Skin. Histometric studies of the activity of the sebaceous glands. First Communication)

Faessler, R. und Krebs, A. (Bern): Spätresultate von bestrahlten Plantarwarzen (Long-Term Reexamination of Plantar Warts Treated by Roentgen Therapy)

Rudzki, E. and Baranowsk Elżbieta (Warsaw): Contact Sensitivity in Stasis Dermatitis

Farah, F.S.; Matta, M.T.; Zeinoun, S., and Shehadeh, S.I. (Beirut): Squamous Cell Carcinoma and Keratoacanthoma in Discoid Lupus erythematosus

Gisslén, H.; Hersle, K., and Mobacken, H. (Gothenburg): Topical Treatment of Cutaneous Candidiasis with 5-Fluorocytosine Compared with Nystatin

Caro, I. and Frootko, J. (Johannesburg): Elastosis Perforans Serpiginosa with Truncus Arteriosus. Case Report

Groot, W.P. de and Wuite, J. (Amsterdam): Livedo Racemosa-Like Photosensitivity Reaction during Quinidine Durettes Medication

The Interesting Case · Der interessante Fall · Un cas intéressant

Velde, J.L. van (Leiden): A Case of Bullous Reticulosis

Exss, R. und Weber, H.-P. (Bonn): Zur Dermopathie bei Phenylketonurie (Reticulosarcoma-Like Skin Lesions in Phenylketonuria)

Book Reviews · Buchbesprechungen · Livres nouveaux
Subject Index
Authors Index
Index Vol. 148

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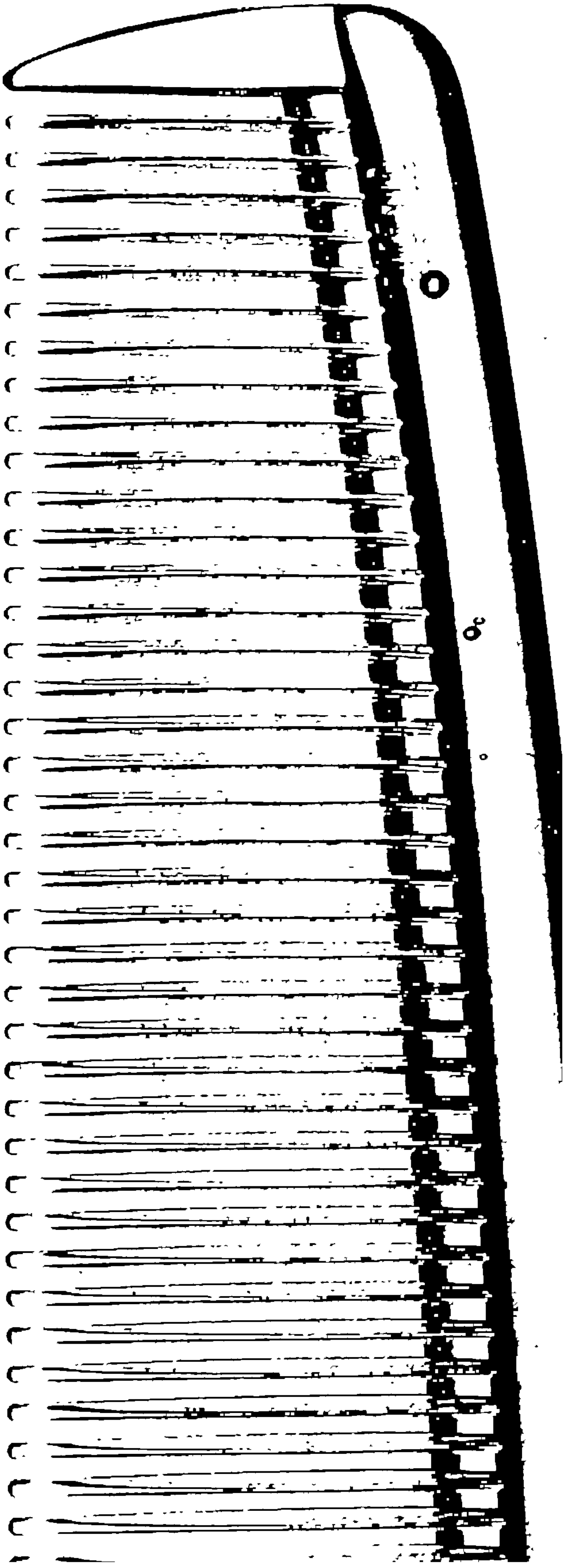

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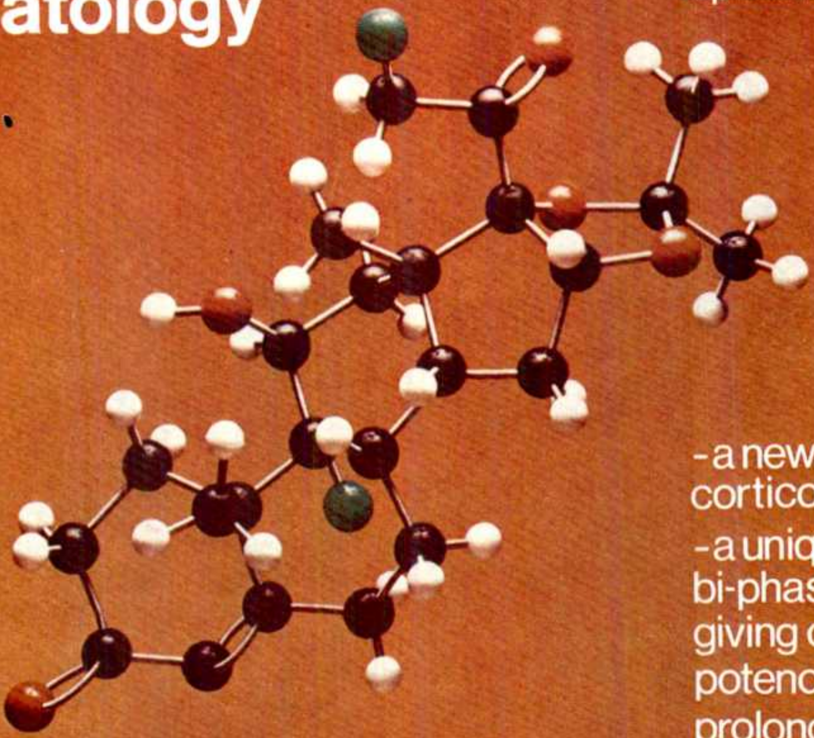

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1. *Practitioner* (1970) **205:** 452.
2. *Brit. J. Derm.* (1968) **80:** 791.
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4. *Brit. J. Derm.* (1965) **77:** 163
5. *Brit. med. J.* (1967) **4:** 535

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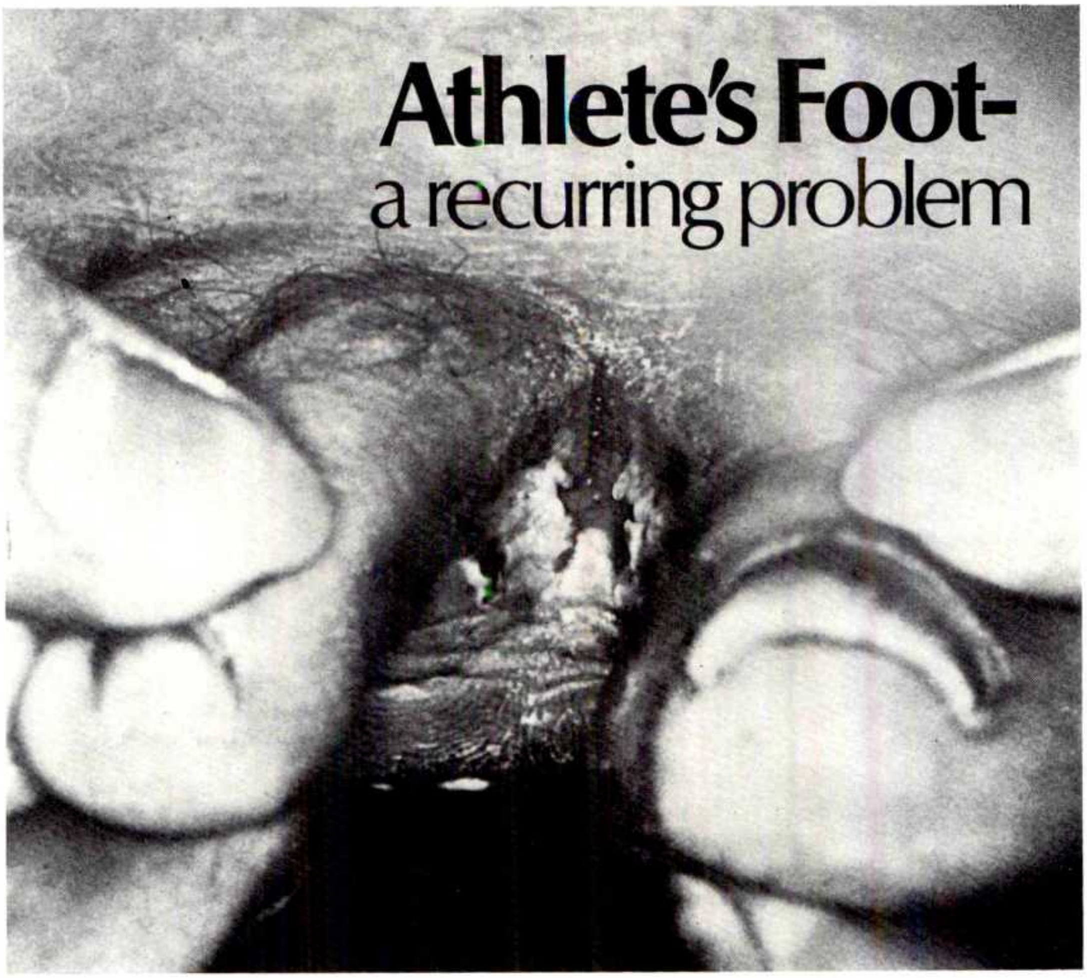

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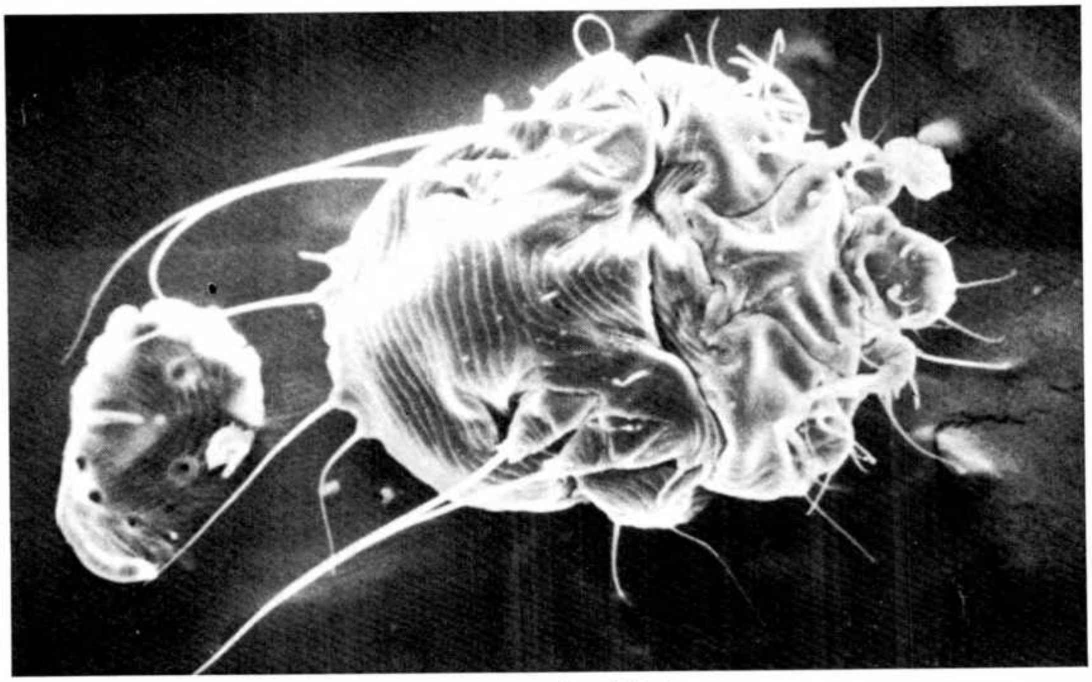

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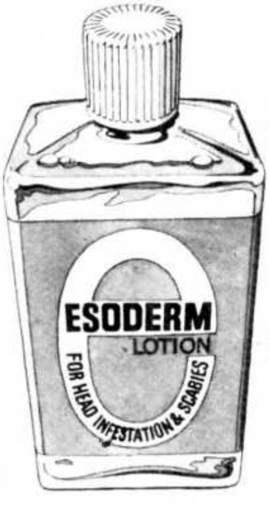

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British Journal of Dermatology (1974) **91**, 365.

Clinical and Laboratory Investigations

Cytodiagnostic use of Feulgen–DNA measurements in cell imprints from the skin of patients with mycosis fungoides

W.A.VAN VLOTEN, P.VAN DUIJN AND A.SCHABERG

Department of Dermatology, Department of Histochemistry and Cytochemistry,
and Laboratory for Pathology, University of Leiden, Leiden, The Netherlands

Accepted for publication 17 January 1974

SUMMARY

To obtain objective criteria for the diagnosis of incipient mycosis fungoides, Feulgen–DNA cytophotometry was carried out in skin imprint preparations from patients with clinically definite mycosis fungoides and from patients suspected of having this disease. Cell imprints of benign skin lesions such as chronic dermatitis served as controls.

All patients with mycosis fungoides showed abnormal histograms with aneuploid and polyploid Feulgen–DNA distribution patterns. In the control group the mean class of Feulgen–DNA values was diploid.

Five per cent or more of non-diploid cells was taken as a criterion for malignancy. On this basis, eight of the fifteen patients in the suspect group showed abnormal histograms. Six of these eight patients developed a malignant skin reticulosis within a year. The other (seven) patients in this group showed normal histograms, and none of them developed a skin malignancy during a follow-up period of 2½ years.

Feulgen–DNA cytophotometry may therefore be considered an objective aid in the diagnosis of the early stages of mycosis fungoides.

The clinical and histological diagnosis of mycosis fungoides remains difficult, despite the many studies carried out to determine the natural history of this disease (Bluefarb, 1959; Epstein *et al.*, 1972).

Possible premycotic lesions have been described under various designations, such as a non-specific erythematous stage, parapsoriasis en plaques, and poikiloderma atrophicans vasculare (Samman, 1964, 1972; Wolff & Selmanowitz, 1970). The biological significance of the classification of these lesions is still controversial, and more objective methods will have to be applied to characterize them.

Feulgen–DNA cytophotometry has been successfully applied to study malignant conditions (Leuchtenberger, Leuchtenberger & Davis, 1954; Atkin, Mattinson & Baker, 1966). There is now general

---

Reprint requests to Dr W.A.van Vloten, Department of Dermatology, Academisch Ziekenhuis Leiden, Leiden, The Netherlands.

agreement that neoplastic cells may contain an abnormal number of chromosomes, resulting in an abnormal nuclear DNA content. These abnormalities are found so consistently that they can be considered an important criterion for malignant growth. Abnormal DNA content of the nuclei has been demonstrated in cells present in precancerous lesions of the breast (Sachs, 1971; Ludwig *et al.*, 1973), cervix (Sandritter, Lobel & Kiefer, 1964), and skin (Kint, 1963; Ehlers & Stephan, 1972).

These findings suggested that similar abnormalities might be found in mycosis fungoides. The purpose of this study was to obtain information about the Feulgen–DNA distribution in lesions of patients with mycosis fungoides and to determine whether such information has diagnostic value in the early stages of malignant skin reticulosis.

MATERIALS AND METHODS

Three groups of patients were examined:

(1) A control group consisting of eight patients with non-malignant skin diseases such as psoriasis vulgaris, chronic dermatitis, and lichen planus.
(2) A group of ten patients with clinically and histologically demonstrated mycosis fungoides in the plaque and tumour stages.
(3) A group of fifteen patients clinically and histologically suspected of a malignant skin reticulosis, e.g. erythroderma and parapsoriasis en plaques.

After disinfection of the skin with chlorhexidine solution and local anaesthesia with 2% lignocaine (Xylocaine®, Astra-Blomberg N.V., Rijswijk, The Netherlands), a skin sample was taken with a 5 mm biopsy punch. Imprint preparations were made with the fresh-cut surface of these skin specimens, and were fixed for 1 h at room temperature in a mixture of methanol, formaldehyde 35% w/v solution (Merck, Darmstadt, West Germany), and glacial acetic acid (85:10:5 volume parts), according to Böhm *et al.* (1968).

To stain DNA, a slightly modified Feulgen procedure was used (Duijndam & van Duijn, 1973). Hydrolysis in 5 N HCl and staining with the Schiff reagent were both performed for 1 h at room temperature. After rinsing in sulphite solution and dehydration, the preparations were mounted in Caedax N_D 1·55 (Merck, Darmstadt, West Germany).

The photometric measurements of the cell nuclei were made with a Zeiss Cytoscan (Zeiss, Oberkochen, West Germany) interfaced to a PDP 12 computer (Digital Equipment Corporation, Maynard, Massachusetts, U.S.A.). The microscope stage was scanned by means of two stepping motors at a speed of 200 steps of 0·5 μm per second. A Neofluar 100/1·30 objective together with a circular measuring diaphragm with a diameter of 0·5 mm was used for the measurements. The actual measuring diaphragm was 0·65 μm when calculated back to the preparation. Measurements were carried out with light of 561 nm, which was selected from a quartz halogen lamp (Philips 12 V, 100 W, 7023) with an interference filter PAL 561 (Schott).

Measurements of the total intergrated absorbances of the Feulgen-stained nuclei were performed with the Fastscan computer programme (van der Ploeg *et al.*, to be published). To obtain these integrated absorbances with appropriate background corrections, the following operations have to be carried out. With the aid of a joystick, the microscope stage is directed to a position where the upper and left boundaries of the cell nucleus touch the image of the ocular cross-hairs. This position is entered into the computer by pressing the marking button of the joystick, whereupon the cell nucleus is moved until the right and the lower boundaries of the nuclear image touch the image of the cross-hairs. With this new position marked, the right-angled measuring area is defined. Two unstained areas at both sides are added to the measuring field as background areas. A test run shows whether the total field to be scanned is free of other particles, and after focusing of the preparation and adjustment of the photomultiplier signal, automatic scanning is started.

The program is designed to distinguish 500 levels of light intensity. To avoid contamination of the results by measurements of nuclear fragments and small artifacts, it will reject a scan and produce a warning message on the VR 12 display when there is more than 10% difference in light intensity between two signals in the background of one scan line. At the end of each scan line, absorbances from the measuring points in the two background areas are averaged to give a mean background value for that line, and each reading on the line within the measuring field is corrected for that background value. At the end of the scan the corrected density values are integrated and the total absorbance of the cell nucleus is typed out by the teletype. The total absorbance per cell nucleus resulting from the scanning measurements gives the total amount of chromophore in the field. Except for possible deviations from the stoichiometry of the DNA dye binding, this amount is a measure for the DNA content of the cell. Sixty cells on two or three slides from each skin sample were measured at random without reference to the cell type.

A computer programme driven–plotter produces a histogram of the distribution of the DNA content of the measured cells. A histogram of sixty cells could be obtained within 90 min. This procedure has the advantage that the total absorbances are measured with great accuracy, because individual background corrections are performed for each cell.

To correct for possible differences due to variations in the Feulgen procedure, smears of chicken erythrocytes fixed in the same way and stained simultaneously with the patients' imprints were used as controls. During the experiments described here the absorbance measurements in the chicken erythrocytes did not differ significantly, so that no corrections were necessary. For histological examination, Haematoxylin and Eosin-stained paraffin sections were made from the same skin samples.

RESULTS

Histology and cytology

Both the histological sections and the cytological preparations of skin from patients in the control group showed infiltration by normal lymphocytes and histiocytes.

In cases clinically diagnosed as definite mycosis fungoides, the sections and imprint preparations showed a polymorphous infiltrate with large atypical histiocytes and reticulum cells, the so-called mycosis cells. Moreover, an inflammatory infiltrate with normal lymphocytes, histiocytes, eosinophils, and plasma cells was present.

Cytological and histological examination of skin biopsy samples from patients in the clinically suspect group revealed some slightly atypical cells in a cell population of normal lymphocytes and histiocytes. The histological pictures raised some suspicion of malignancy, but a final conclusion could not be reached.

Cytophotometry

In Table 1 the percentages of cells in the DNA classes are given for each group of patients. Representative histograms of each group are shown in Fig. 1.

The Feulgen–DNA values of all patients in the control group were restricted to the diploid region. The frequency of tetraploid cells was less than 4% in all cases. Tetraploid Feulgen–DNA values occur in the cells preparing to divide. These normal histograms were used as standards for the interpretation of the other histograms.

All Feulgen–DNA histograms of patients with clinically definite mycosis fungoides were abnormal. They showed a distinctly aneuploid and polyploid distribution of the Feulgen–DNA values, ranging

TABLE 1. Feulgen–DNA distribution in percentages calculated in the three groups of patients

Patient no.	No. of cells	DNA histogram (%)						
		< 2n	2n	3n	4n	6n	8n	> 8n
Patients with benign skin lesions								
40	60		98·3			1·6		
48	50		100					
49	41	2·4	97·5					
50	60		96·6	1·6	1·6			
51	60		100					
66	60		98·3		1·6			
89	60		95	1·6	3·3			
102	60		96·6		3·3			
Patients with mycosis fungoides								
31	60		73·3	3·3	16·6	5	1·6	
32	60		73·3	8·3	18·3			
42	60		25	10	60	5		
56	60		10	66·6	3·3	20		
62	60		76·6	13·3	10			
67	60		25	5	58·3	8·3	3·3	
68	60		55	3·3	26·6	10	3·3	1·6
70	60		78·3	8·3	11·6	1·6		
75	60		51·6	20	23·3	5		
78	60		85	8·3	1·6	5		
Patients suspected of malignant skin reticulosis								
64	60		81·6	3·3	15			
83	60		90	5	5			
74	60		86·6	6·6	6·6			
84	60		78·3	18·3	3·3			
65	60		91·6	1·6	6·6			
73	60		80	13·3	5	1·6		
76	60		66·6	23·3	10			
109	60		78·3	6·6	15			
53	60		98·3		1·6			
36	60	15	81·6	1·6	1·6			
79	60	5	88·3	3·3	3·3			
39	60		91·6	6·6	1·6			
58	60		96·6		3·3			
110	60		91·6	5	3·3			
34	60	5	95					

from diploid to octoploid and beyond.

Abnormal histograms were obtained in eight out of fifteen patients in the suspect group. These abnormal histograms showed a frequency of 5% or more of cells in the tetraploid and higher ploidy classes. Six of these patients developed a malignant reticulosis of the skin within a year. The follow-up period of two other patients with an abnormal histogram is still too short. Seven patients in the suspect group showed normal histograms, and none of them developed a malignant skin reticulosis during a follow-up period of 2½ years. Table 2 gives the initial diagnosis, the Feulgen–DNA histogram and the most recent diagnosis.

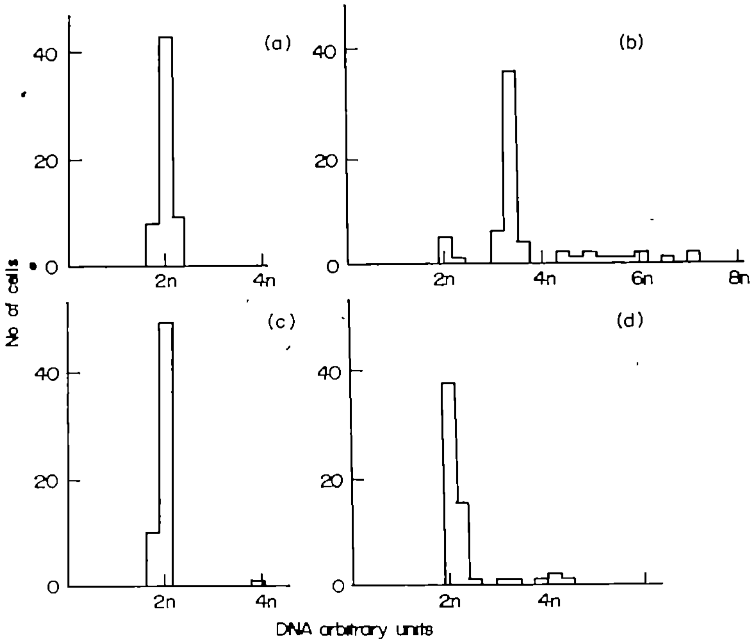

FIGURE 1. Feulgen–DNA histogram of four representative patients. (a) patient no. 51, chronic dermatitis; (b) patient no. 56, mycosis fungoides; (c) patient no. 53, suspected of malignant skin reticulosis, diagnosed after 22 months as seborrhoeic eczema; (d) patient no. 74, suspected of mycosis fungoides, diagnosis confirmed after 17 months.

TABLE 2. Relationship between clinical diagnosis and DNA histogram in patients suspected of malignant skin reticulosis

Patient no.	Initial diagnosis	DNA histogram	Follow-up (months)	Recent diagnosis
64	Mycosis fungoides ?	Abnormal	19	Mycosis fungoides
83	Mycosis fungoides ?	Abnormal	3	Mycosis fungoides
74	Mycosis fungoides ?	Abnormal	17	Mycosis fungoides
84	Erythroderma	Abnormal	11	Malignant reticulosis
65	Erythroderma	Abnormal	19	Sézary syndrome
73	Parapsoriasis en plaques	Abnormal	14	Mycosis fungoides
76	Parapsoriasis en plaques	Abnormal	13	Parapsoriasis en plaques
109	Mycosis fungoides ?	Abnormal	5	Mycosis fungoides ?
53	Mycosis fungoides ?	Normal	22	Seborrhoeic eczema
36	Mycosis fungoides ?	Normal	32	Atopic eczema
79	Erythroderma	Normal	17	No skin disease
39	Erythroderma	Normal	25	Psoriasis vulgaris
58	Parapsoriasis en plaques	Normal	20	Parapsoriasis en plaques
110	Parapsoriasis en plaques	Normal	5	Parapsoriasis en plaques
34	Parapsoriasis en plaques	Normal	32	Parapsoriasis en plaques

DISCUSSION

Most human tissues are made up of diploid cells with a fixed chromosome complement and a constant amount of DNA. Cells with an increased number of chromosomes, and therefore an increased amount of nuclear DNA, are occasionally present as the result of mitotic irregularities such as are seen in tumour cells. In the enhanced proliferation occurring in chronic inflammation too, a few cells in the interphase may have DNA values lying between the diploid and tetraploid values.

In our control group of patients with chronic dermatitis, psoriasis vulgaris and lichen planus, the proportion of hyperdiploid and tetraploid cells was maximally 4% of the total number of measured cells. It was impossible to obtain skin-imprint preparations from entirely normal skin without any signs of inflammation, because all the cells in the dermis are so entrapped in the collagen. On the basis of these investigations we considered a frequency of 5% of tetraploid and higher ploidy cells as abnormal.

The cytophotometric results obtained in patients with definite mycosis fungoides showed great variation, with up to 60% of the cells beyond the diploid region. This is in agreement with earlier cytochemical studies on human malignant tumours (Adams & Dahlgren, 1968; Stich & Steele, 1962; Manocha, Steele & Stich, 1969; Knoth & Sandritter, 1965).

In our group of fifteen patients suspected of malignant skin reticulosis, eight showed a histogram with 5% or more of the cells in the tetraploid and hypertetraploid region, and seven had histograms with less than 5% of abnormal cells. In the follow-up period none of these seven patients developed any evidence of skin malignancy, whereas six of the eight patients with an abnormal histogram according to our criteria did develop malignant skin reticuloses.

Although the follow-up period in this study was only 2½ years, we conclude that DNA cytophotometry is a method that can contribute to the early diagnosis of mycosis fungoides. In this disease, early diagnosis can be important for the choice of therapy.

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Eczema provoked by methyl dopa

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SUMMARY

Sixteen patients are described in whom seborrhoeic or nummular eczema erupted while they were taking methyl dopa. The eruption only responded to treatment when the methyl dopa was stopped. In eight patients who had recovered, test doses of methyl dopa caused rapid recurrence of eczema. The mechanism is obscure but appears to be provocation of endogenous eczema rather than a true drug eruption.

Eczematous eruptions caused by systemically administered drugs are common enough when the epidermis has been previously sensitized by local application of the drug itself or an analogue, but when they arise during systemic administration only, direct association between the drug and the eruption is difficult to prove.

It is not uncommon to see nummular eczema during the treatment of arthritis with gold, but the provoking mechanism is obscure. Antituberculous therapy with streptomycin, PAS and isoniazid also produces lesions resembling nummular eczema or seborrhoeic dermatitis. Crissey, Osborne & Jordon (1957) studied thirty-seven such patients, and suggested that the first two drugs produced an alteration in the skin bacterial flora which might underlie the eruptions. Phenindione has been blamed by Copeman (1968) for causing an itchy eczema.

Peterkin & Khan (1969) described four patients with eczema of the palms and soles which developed while taking methyl dopa. In two cases the eczema became widespread and in all it persisted until the drug was stopped. Copeman (1972) also recorded four patients with discoid eczema while taking methyl dopa.

Methyl dopa has been in common use as an antihypertensive agent for over a decade. It acts as an inhibitor of the enzyme dopa decarboxylase, reducing the rate of synthesis of catecholamines and other aromatic amines, but invesigations into the pharmacology of the drug show that its hypotensive effect does not appear to be related to enzyme inhibition (Laverty, 1973). There are three other theories as to its mode of action, none of which has been conclusively proven.

(1) That methyl dopa forms false transmitters in sympathetic nerve endings which replace and are released instead of noradrenaline but which have a less potent action.

(2) That methyl dopa has a direct peripheral vasodilator effect (Mohammed *et al.*, 1968).

(3) That methyl dopa is converted to a methyl noradrenaline and acts in the CNS by stimulating adrenergic receptors (Henning & Rubenson, 1971).

In the past 4 years sixteen patients have been seen suffering from eczema, usually of the seborrhoeic distribution, less often nummular, which erupted while they were taking methyl dopa.

CASE REPORTS

The following brief case histories give the clinical picture.

Case 1

A.B.W., a male aged 57, suffered from nephritis at the age of 21 and developed hypertension and uraemia at 53 years of age. Treated with methyl dopa 250 mg three times daily, within a few weeks he developed eczema of the feet, legs and hands which was partly controlled by local steroids. In 1969 eczema spread to involve the scalp, face and trunk. Admission to hospital and local steroid therapy produced no improvement in 2 weeks. Methyl dopa was therefore stopped and other antihypertensive drugs substituted. In 3 days the skin healed and itching ceased. Methyl dopa was then recommenced. In 24 h the itching recurred and in 2 days the eczema reappeared. Methyl dopa was again stopped, hydrallazine was substituted and in a week the skin healed.

Case 2

L.W., a woman aged 72, 3 years after starting methyl dopa developed a rash under the breasts, which rapidly became generalized and particularly scaly and itchy in the scalp (Fig. 1). She was admitted to hospital and methyl dopa was stopped. She remained normotensive. Within 17 days the skin healed. A test dose of 250 mg of methyl dopa daily caused a recurrence of the eczema in a week. She remained well after the drug was again stopped.

Case 3

H.D., a male aged 51, had been on methyl dopa for 6 months and for 1 month had suffered a characteristic seborrhoeic eczema of the scalp, face (Fig. 2), neck, axillae, antecubital fossae and thighs. The drug was stopped and the skin healed in a few days. Administration of the original dose of methyl dopa 250 mg four times daily was followed 24 h later by swelling, redness and scaling of the pinnae and itching of the scalp. As he was severely hypertensive, debrisoquine was substituted and he remained well.

Case 4

P.G., a woman aged 64, had a history of eczema in infancy and asthma. For 18 months she had taken methyl dopa and had suffered increasingly severe eczema, especially of the scalp from which she had lost much of her hair (Fig. 3). Three weeks after admission to hospital and stopping the drug her skin had almost healed. She was given inert tablets resembling methyl dopa for 3 days without effect. Methyl dopa 250 mg four times daily was then recommenced and in 48 h the scalp again became bright red and itchy. Other antihypertensive drugs were substituted, her eczema has healed and the hair regrown.

Case 5

J.D., a woman aged 45, developed a discoid eczematous eruption on the face, neck and limbs within 2 weeks of starting to take methyl dopa. She also had a strongly positive capillary fragility test. Both reactions healed when guanethidine was substituted for the methyl dopa. Because of severe hypertension, alteration of drugs for test dosing was not considered justifiable.

Eleven more patients have been seen whose clinical features are summarized in Table 1. It will be

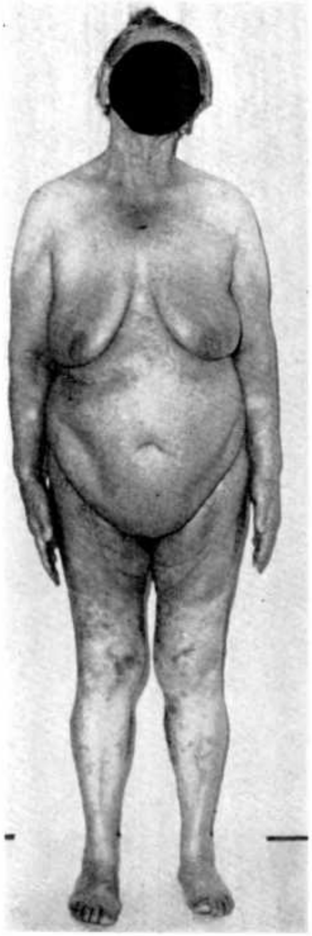

FIGURE 1. Case 2. Widespread seborrhoeic eczema.

seen from Table 1 that of sixteen patients, fifteen were taken off methyl dopa and where necessary another antihypertensive drug was substituted. All recovered completely. One patient continues on methyl dopa because other antihypertensives are considered unsuitable as she has polycystic kidneys. Her eczematous eruption is still persistent but partly controlled by local corticosteroids.

In patients with severe hypertension it is not always easy to justify frequent changes in therapy but it has been possible to give test doses of methyl dopa to eight patients. The skin eruption recurred within a day or two when full doses were used, but if smaller doses were given, over 1 week's administration was necessary to produce a relapse. All relapsed except one patient whose test doses were probably too small and too brief. A ninth patient resumed methyl dopa after her skin had been clear for 6 months and had no further trouble. If the epidermis had been sensitized by some unknown analogue, methyl dopa itself might produce a positive patch test. However, patch tests with a 1% aqueous solution produced no reaction in six patients.

One of the interesting side effects of methyl dopa therapy is the development of an autoimmune haemolytic anaemia. In such patients an IgG antibody can be demonstrated attached to the patient's red cells and showing specificity for the Rhesus locus. A positive Coombs' test can be demonstrated

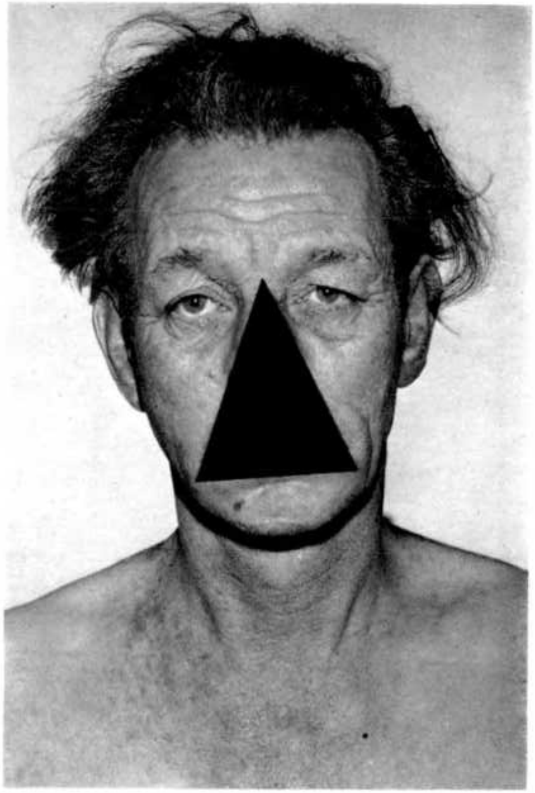

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FIGURE 2. Case 3. Seborrhoeic eczema.

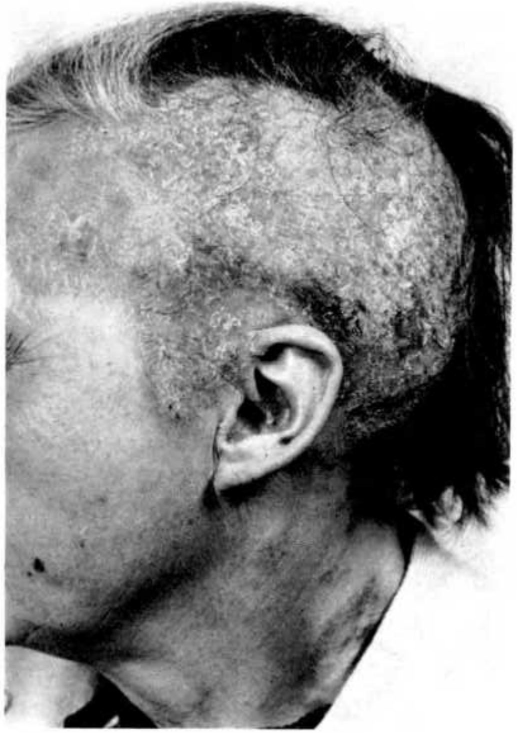

FIGURE 3. Case 4. Atopic eczema.

TABLE 1. Summaries of 11 more case histories

Patient	Sex	Age (years)	Period on methyl dopa	Duration of eczema (weeks)	Site of eczema	Healed on stopping drug (weeks)	Test dose	Previous disease
6. J.K.	M	51	24 months. 2 months dose increased	6	Scalp	4		
7. S.W.	M	56	60 months		Scalp, face, trunk	12		Seborrhoeic dermatitis
8. N.H.	F	67	? years	5	Scalp, face	4		
9. P.W.	F	76	24 months	12	Generalized	2	Positive	
10. M.A.	F	74	36 months	16	Scalp, trunk	1	Positive	
11. M.F.	F	79	10 months	8	Generalized	2	Negative	
12. I.H.	F	69	8 years	52	Scalp, trunk	6		Hair dye dermatitis
13. H.W.	F	63	3 years, 1 year dose increased	52	Scalp, arms, trunk	Drug continued		Ichthyosis
14. F.M.	F	76	36 months	16	Generalized	1	Positive	Eczema
15. L.O.	F	71	4½ years	20	Scalp, limbs	3		
16. K.B.	F	62	2 years	12	Trunk, limbs	3		Ichthyosis

before there is clinical evidence of anaemia. About 20% of patients on methyl dopa develop a positive direct Coombs' test. The incidence rises with increasing dosage and appears between 6 and 12 months after treatment begins. Two such patients have also been described with positive LE cell tests and rheumatoid factor (Sherman, Love & Harington, 1967).

These immunological responses do not appear to be linked to the skin eruption; in Copeman's cases (1972) Coombs' test was negative and the immunoglobulins normal, while in the present series five patients have been similarly investigated and Coombs' test found positive in only one, which is the expected incidence.

What does seem significant is that five of these sixteen patients had a history of previous skin trouble. In one this was infantile eczema, two others had also had eczema years before, one seborrhoeic dermatitis and one 20 years previously had had hair dye dermatitis. A sixth patient had a recurrence of discoid eczema, apparently provoked by emotional stress 1 year after she had recovered from the methyl dopa-induced attack.

CONCLUSIONS

The epidermal reaction in these patients is not an ordinary drug eruption but appears to be a provocation of eczema by the drug. Although more likely to occur in those who have had previous eczema or dermatitis, it may precipitate eczema for the first time in the patient's life. To some extent it is related to dosage, as in two cases the eczema broke out within a week or two of an increase in the dose of the drug, and in test dosing relapse occurred swiftly on full doses but only after many days when less than the usual theraputic dose was given.

A possible mechanism is the vasodilator effect of the drug, but this is unlikely to be the complete explanation as some of the anti-hypertensive drugs which were successfully substituted for methyl dopa are also vasodilators.

Eczema is not the only endogenous epidermal reaction to be exacerbated by systemically administered drugs, as psoriasis may be made worse by administration of chloroquine or quinidine. It seems possible that gold, streptomycin, PAS, phenindione and methyl dopa are not the only drugs which provoke eczema and it might be rewarding to look for others in patients whose eczema resists treatment.

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The spectrum of paediatric dermatology

ANALYSIS OF 10,000 CASES

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SUMMARY

Almost 10,000 paediatric dermatological cases seen as out-patients were classified according to diagnosis, sex and age. Diagnostic frequencies, relationships between diagnostic categories, biological significance of high and low frequencies and disturbances of sex ratios are noted. The application of this knowledge to medical education is outlined.

Until some 15 years ago, no one could say what proportions of skin diseases occurred in the Transvaal. Medical and dermatological services existed, but their practise was often unkempt and traditionless. Particularly was this so in dermatology. Charting the wilderness by making surveys of skin patients in bulk was started by us in 1956. This attempt issued in a series of publications: 600 Bantu skin patients (Findlay, 1957); 180 white infants (Findlay, 1958); 13,500 Whites (Findlay & Scott, 1960); retrospect, pre-1914 (Findlay, 1961); 4000 Bantu (Schulz, Findlay & Scott, 1962); age incidences in 16,500 White patients (Findlay, 1967); age incidences in 4500 Bantu (Park, 1968); age incidence patterns in White, Bantu and Indian with 22,000 cases (Findlay & Park, 1969). Our present study covers a field which has not been considered in comparable detail before, and comprises a full year-by-year analysis by diagnosis, sex and age of skin patients up to the age of 12 years. A portion of these findings is reported here.

SCOPE

As opening comment, some general features of our approach may be clarified.

It is still unpractical to make an unbiased survey of the population at risk in order to study the full round of skin diseases. Selection through the influence of personal concern by patient and doctor is easier and more rewarding at present. Rather to our surprise, the large patient samples have proved to contain a roughly proportional representation of most age-groups of the population at risk. This meant that diseases with different age distributions phased in and out so as to leave each age-group with roughly the same net load of skin disorders as any other. In earlier studies, as in the present one, the neonatal period was poorly represented. There was a lull in skin diseases in mid-childhood, and females preponderated.

From a balanced survey, there ought to emerge a confidence with what is known to occur, and a confident recognition of whatever is different or new. A scale of frequency of diagnoses can determine the breadth, and individual discretion the depth, of one's instruction to students. At present there can be no simpler or superior academic principle. One dispenses at once with inadequate systems based on pathology, aetiology, lesion types, 'basic sciences', etc. Any statistical review of the common dermatoses also helps to test the validity of accepted ideas which possibly owe their currency to mere repetition. In making diagnoses we have also focused attention on the content of the complaint, rather than give way unduly to psychological deductions to be made from the patient's manner.

The frequency of a skin condition can sometimes be given a biological interpretation according to the position which it occupies on the frequency-scale in question. This proposition has been argued elsewhere (Findlay, 1974b), but briefly it is as follows: the more frequent disease-pictures are of recent evolutionary origin, and involve complex, semi-adaptative changes on the wave-front of evolution in the organism and its environment; contrariwise, the rarer the disease picture, the more it represents the crisper, bizarre and non-adaptative changes which are related to biological faults in well-established processes that were developed further back in evolutionary history.

Surveys of the present type also illustrate the balance of demand and supply in specialist services. Furthermore, today's providers of therapeutic measures (drug houses, etc.) require information of this sort on the frequency, severity and course of complaints, to gauge the market for products, both old and new.

MATERIAL

From an initial total of nearly 10,000 cases, 9877 white out-patients up to the age of 12 seen both in hospital and in private practice were found suitable for the present survey. They had been seen by four dermatologists with similar diagnostic habits. Approximately 5600 were seen by G.H. Findlay (Pretoria), 2200 by D.A. Whiting (Johannesburg) and 1450 and 560 by E.J. Schulz and T. Heyl respectively (Pretoria). Almost all the cases surveyed here were seen through referral by a colleague—general practitioner, hospital medical officer, paediatrician, etc.—who wished to have the case treated by a dermatologist.

In the present analysis, only the first two diagnoses and the patient's age and sex were extracted. The age represented the time of the first visit when the complaint was active, regardless of its period of onset or later duration. This information was interpreted as the liability to have active disease of a given type within the limits of age, sex and population group considered. Diagnostic categories were sorted according to the coding system advocated by Calnan & Meara (1957), which we have found adaptable and useful ever since it was published.

RESULTS

Age and sex distribution in patient sample (Fig. 1)

It is apparent from the distribution chart that, in childhood, girls are seen progressively more often than boys as they grow older, suggesting perhaps an increasing concern for the skin of the growing girl. All ages are well represented, though one can judge that skin diseases are a greater problem up to the age of 2 than at any later time. There is no obvious sex difference in this infant group. After 5 years of age there is not much fluctuation in the total numbers of cases seen.

Diseases diagnosed (Table 1) and their percentage frequency (Fig. 2)

The percentage of each diagnosis in the sample as a whole, given in Table 1, exceeds 100% by virtue

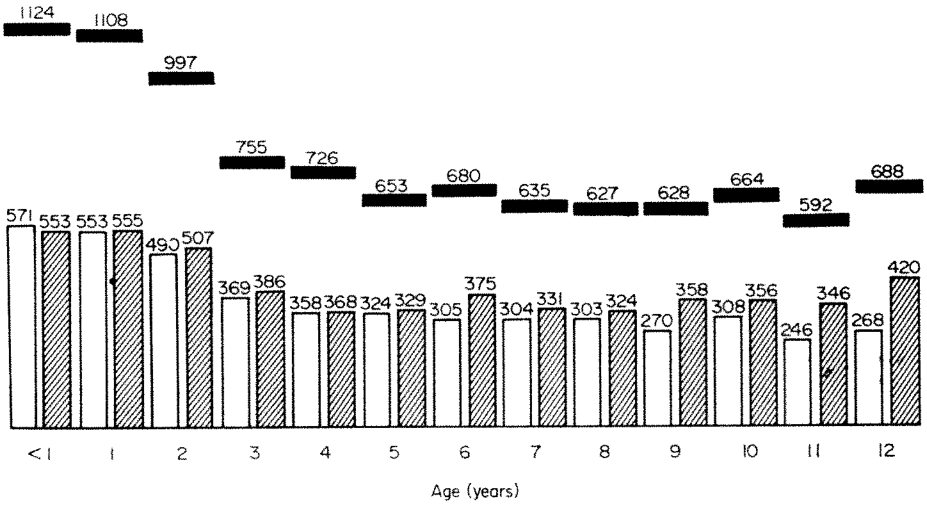

FIGURE 1. Age and sex distribution of the paediatric skin cases studied. Open columns, male; hatched columns, female.

of 15% of dual diagnoses. Taking the diagnoses in declining order of frequency, Fig. 2 graphs the number of diagnoses (y) which will take care of any given percentage of paediatric skin cases (x).

In planning for general practice for example, one can decide how far down the list of diagnostic frequencies the practitioner should find it worth his while to study. Obviously the decision depends on the expected proportion of diagnoses falling within the scope of paediatric dermatology, and how long on the average one wishes to wait before encountering one case with a particular diagnosis.

As an example, let a diagnosis be considered worth making even when the condition turns up only once in 5 years. If a general practice profile contains 8% dermatology with a proportionate representation of all age groups, about 25% of these will then be children aged 12 and under, i.e. 2% of the total diagnoses. Taking granuloma annulare, which commonly falls near the boundary of a general practitioner's knowledge, it has a paediatric skin incidence of 1%, so that in our model practice it will turn up in 0·02% of cases, or 1 : 5000. Over 5 years this means a minimum of 1000 diagnoses of all kinds per year if it is to turn up once as anticipated. In this way one can offer a concrete justification for a syllabus, in place of the inclination to pontificate in the choice of diseases to be studied.

Sex distribution among diseases in general

For most of the commoner diseases in our series there was no obvious difference in sex incidence. A large difference was noted in seven major diagnostic categories, and these are shown in Table 2.

Unequal sex ratios for a given disease may depend on differences in the threshold of concern was ell as differences in frequency and severity. Some of these inequalities may be traceable to sex differences in behaviour and fashion. A more venturesome disposition out of doors would expose boys more readily to larva migrans, tinea capitis and animal ringworm generally, and their short scalp hair would further increase the chance of tinea capitis. Scabies, which spreads best in a household environment, could thereby affect girls for preference, who are more inclined to stay indoors.

Of the four diagnoses where girls outweigh boys in numbers (acne, psoriasis, granuloma annulare and angiomas), there is doubtless a blend of heightened concern for the girl with a greater tendency to the complaint.

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TABLE 1. Percentage incidence of diagnoses in skin out-patients aged 12 years and under in declining order of frequency (note the overlap of several categories)

Diagnosis	Percentage	Diagnosis	Percentage
Eczema—constitutional—atopic	18·7	Verruca vulgaris	10·7
1–10%			
Impetigo	7·8	Psoriasis	2·0
Dermatitis, unspecified	6·1	Eczematide	1·9
Tinea capitis	5·1	Alopecia areata	1·6
Dermatitis, seborrhoeic	3·4	Verruca plana	1·5
Lichen urticatus (papular urticaria; insect bites)	3·2	Xerosis	1·5
Pityriasis alba, simplex	3·1	Pityriasis rosea	1·3
Larva migrans	2·9	Acne, infantile, and acne vulgaris	1·2
Scabies	2·8	Candidiasis	1·1
Tinea corporis	2·4	Molluscum contagiosum	1·1
Haemangioma, all types	2·4	Granuloma annulare	1·1
Plantar warts	2·3	Ichthyosis	1·0
Eczema, hand–foot	2·1	Eczema, nummular	1·0
0·5–0·9%			
Urticaria	0·9	Boils, carbuncles	0·5
Vitiligo	0·8	Contact dermatitis	0·5
Naevi, various linear	0·7	Ecthyma	0·5
Erythemas, including scarlet fever	0·7	Herpes simplex	0·5
Myiasis, insect bites	0·6	Naevus cell naevi	0·5
Keratosis pilaris	0·6	Mastocytosis	0·5
Erythema multiforme	0·6		
0·2–0·4%			
Prurigo, all types	0·4	Xanthoma, naevoxantho-endothelioma	0·2
Naevi, epidermal	0·4	Epidemic follicular keratosis	0·2
Kerion	0·4	Geographical tongue	0·2
Keratolysis exfoliativa	0·4	Onychia, paronychia	0·2
Tylosis	0·4	Nail dystrophies	0·2
Hyperhidrosis	0·4	Naevi (other linear types)	0·2
Gianotti–Crosti syndrome	0·4	Lichen planus	0·2
Folliculitis	0·4	Herpes zoster	0·2
Dermatitis, napkin	0·4	Hairfall	0·2
Hypomelanoses—all types	0·4	Granuloma pyogenicum	0·2
Actinic dermatitis	0·4	Drug eruption	0·2
Comedone formation	0·4	Cysts	0·2
Sporotrichosis	0·3	Cheilitis	0·2
Lichen striatus	0·3	Abscess	0·2
Septic dermatitis, not classified elsewhere	0·3		

0·1% (selected examples from a total of fifty-four categories containing about nine cases each)

Arbovirus infections; Allergide; Adenoma sebaceum; Chilblains; Dermatitis herpetiformis; Other miscellaneous types of dermatitis; Epidermolysis bullosa; Erythroderma, congenital ichthyosiform; German measles; Hirsutism; Incontinentia pigmenti; Kaposi varicelliform eruption; Lichen sclerosus; Lipoid proteinosis; Lymphangioma; Lupus erythematosus; Morphoea; Neurofibroma; Porphyria; Parapsoriasis, all types; Pityriasis rubra pilaris; Pityriasis versicolor; Striae; Telangiectases; Vaccinia; Varicella

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0·04% (selected from ten categories, four cases each)

Acanthosis nigricans; Pseudoxanthoma elasticum; Scleroedema

0·03% (selected from eleven categories, three cases each)

Bilharziasis; Dermatomyositis; Pemphigoid

0·02% (selected from twenty-three categories, two cases each)

Lymphoedema; Ehlers-Danlos syndrome; Hand, foot and mouth disease; Acrodermatitis enteropathica

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0·01% (selected from twenty-eight categories, one case each)

Darier's disease; Squamous epithelioma; Fat necrosis; Eosinophilic granuloma; Porokeratosis; Confluent and reticulated papillomatosis; Leprosy; Tuberculosis; Reticulosis

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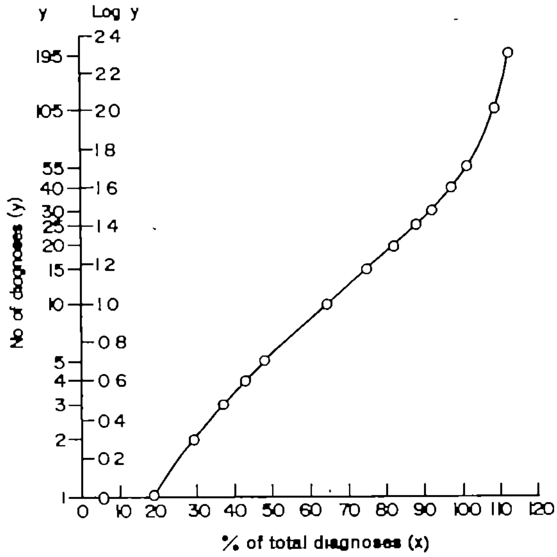

FIGURE 2. Graph showing how many diagnostic categories in order of frequency must be known (y axis) in order to cover any chosen percentage of paediatric skin patients seen (x axis). The order of frequency is found in Table 1. The total percentage is over 100% because of double diagnoses.

TABLE 2. Major diagnoses (over 100 cases) showing obviously unequal sex ratios : comparison with patient and population patterns

Diagnosis	No. of cases	Sex ratio (M : F)
Acne	154	36 : 100
Angiomas—all types	233	52 : 100
Granuloma annulare	108	66 : 100
Psoriasis	197	62 : 100
Larva migrans	282	100 : 67
Scabies	277	65 : 100
Tinea (capitis, corporis, kerion)	783	100 : 63
Patient population (present study)	10,000	90 : 100
Population at risk (ages 0–14)	Census 1960	105 : 100

Age and sex distribution of selected diseases

Acne. Two findings were notable: (1) infantile acne affects male babies earlier and more severely than the female; (2) the 10–12 age period is heavily loaded with acne in females.

Eczema. Relationships between types of eczema have hitherto been based upon degrees of overlap of principally clinical findings. There are many examples of eczema which by this method cannot be readily fitted into a major grouping. Having established that there are no striking sex differences in the paediatric age group of eczemas, the smoothed plots of age incidence show some interesting features. Comparing the figures attributed to the constitutional–atopic group, the 'eczema unclassified' group, the eczematides, and what we have grouped as nummular eczema, the age distribution apparently forms a family of curves (Fig. 3). The constitutional–atopic group is much more frequent and starts earlier in life than the others. At the opposite end of the scale is the nummular group; least widespread and reaching a slightly later peak. Between these poles of morphologically characteristic cases is a large mass of morphologically indeterminate cases, which fit in well as intermediate forms. One might say that the atopic picture gives a high intensity spectrum and that nummular eczema is a low intensity spectrum, with the others in between. The graphs therefore provoke the idea that different clinical patterns may relate to different values of a constant in an equation, rather than to a series of distinguishable but overlapping clinical patterns. What they do not tell us is intensity of what, though one cannot help being reminded of Wien's law of spectral shift in radiation by way of a physical analogy.

On either side of this family of curves stand two others which differ considerably; those of seborrhoeic eczema and hand-and-foot eczema (Fig. 4). The former precedes and the latter follows the 'family', both in intensity and in timing. Perhaps all these differences will in time to come be related to phases of development and control of different areas of the skin.

Psoriasis; verruca vulgaris. These two conditions illustrate the way in which some diseases increase as others wane (Fig. 5). While there has been some debate about the status of infantile psoriasis, it is plain that psoriasis as commonly understood is more inclined to supplant than add to the eczemas. The division between eczema and psoriasis lies roughly between 4 and 6 years of age. From here towards puberty a new population of diseases fills the gap left by the decline of eczema. Psoriasis and verruca vulgaris provide examples of this replacement.

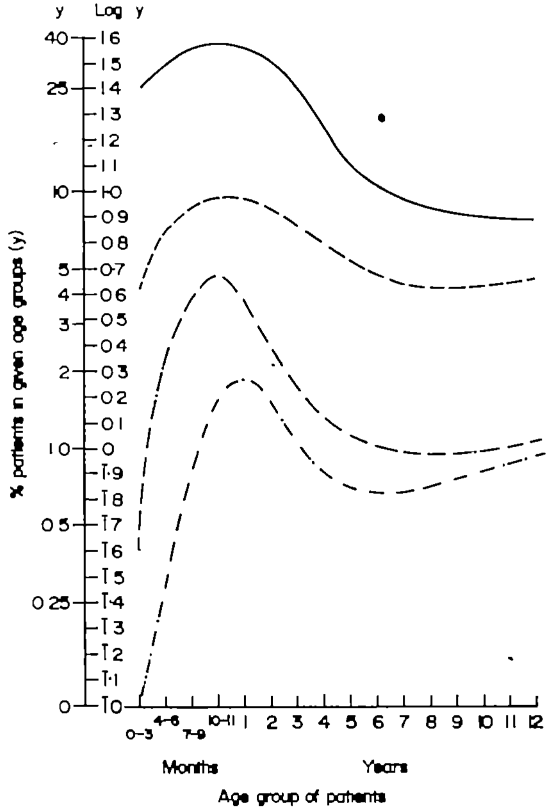

FIGURE 3. Family of eczema curves (age against log percentage frequency) from above down. ———, constitutional atopic eczema; — — —, eczemas 'unclassified'; ———, eczematides; —·—·—, nummular eczema.

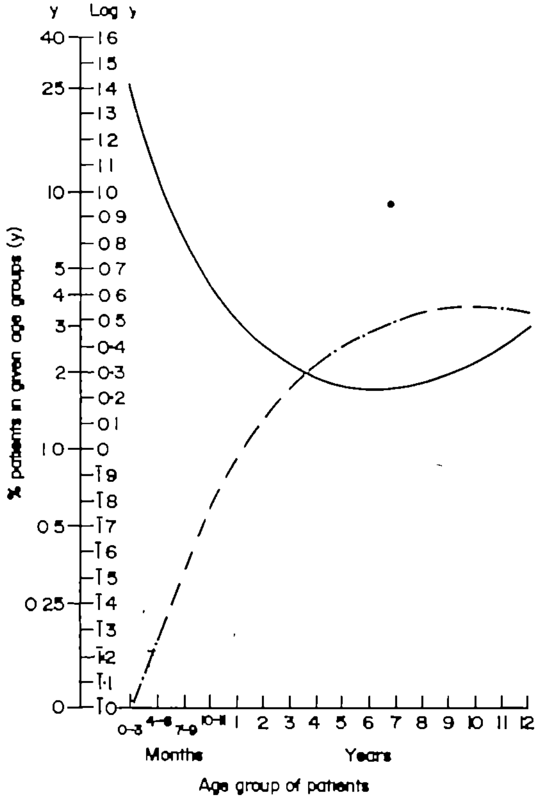

FIGURE 4. Seborrhoeic eczema (——) and hand-and-foot eczema (—·—·—) percentages (log) against patient's age. Smoothed.

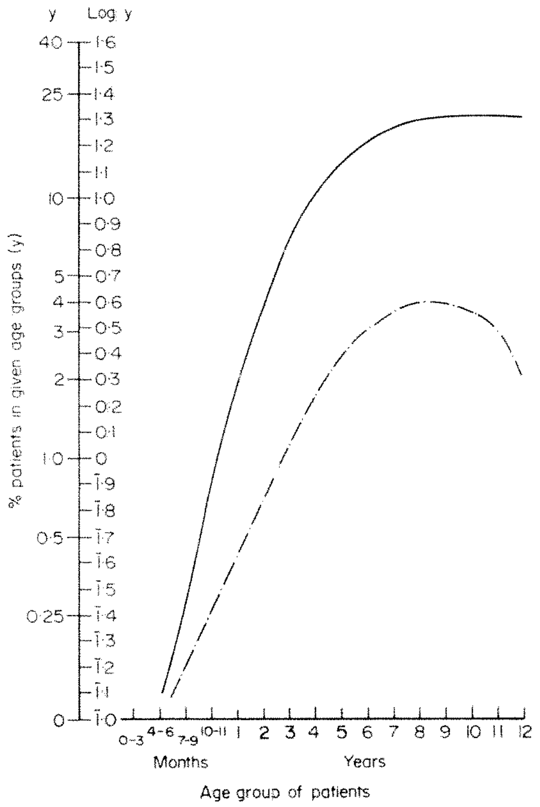

FIGURE 5. Verruca vulgaris (——) and psoriasis (—·—·—) Percentages (log) against age. Smoothed.

Tineas. These have been discussed elsewhere (Findlay, 1974c); the conclusions for tinea capitis caused by *Microsporum canis* are nevertheless worth brief mention. The age peak lies in the period immediately before school age (5–6 years) and declines progressively thereafter until a point of disappearance during puberty. Such a pattern of age incidence combines at least two variables; an interest in animal pets which falls off once the child goes to school and the well known immunity to scalp ringworm which develops at puberty.

ACKNOWLEDGMENTS

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An outbreak of *Trichophyton rubrum* infection in a geriatric hospital

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SUMMARY

The investigation of an outbreak of ringworm affecting fourteen female patients in two wards of a geriatric hospital, due to an unusual strain of *Trichophyton rubrum*, is reported. Most of the affected patients were incontinent and developed skin lesions over the buttocks, the backs of the thighs, or the lumbosacral region.

Trichophyton rubrum, a dermatophyte thought to have been long endemic in the Far East, was noted only sporadically in Western Europe before the First World War. By 1958, St John's Hospital for Diseases of the Skin was registering 100–200 new patients with *T. rubrum* infection each year, not only in troops returning from the Second World War, but also in people who had never been abroad (Calnan, 1958).

In Western Europe at the present time, *T. rubrum* is the most common dermatophyte to be isolated from patients attending dermatological clinics, although *T. interdigitale* infections are more common in the general population. Any part of the skin surface may be affected by *T. rubrum*, but infection of the feet is most common (86%), this usually being the primary site (Rosman, 1966). In India, on the other hand, *T. rubrum* infection is the usual cause of primary body ringworm, a common dermatosis in that country, but infection of the feet is rare.

The present paper reports the investigation of an outbreak of primary *T. rubrum* infection of the body, affecting fourteen female patients in two wards of a 500 bed geriatric hospital.

SUBJECTS AND METHODS

All clinical studies and inspection of the wards were carried out by R.D.G.P.; laboratory work was done by M.P.E.

Two separate female wards were affected. Ward A had a total of twenty-four beds split between a large and a small ward area, while Ward B had twenty patients in a single ward. The lay-out of each ward, with the bed positions of affected patients, is shown in Figs. 1 and 2.

Many patients in the two wards were incontinent, but most sat up in chairs at some time during the day, each using her own chair and draw-sheet. Sheets were changed and sent to the laundry as soon as

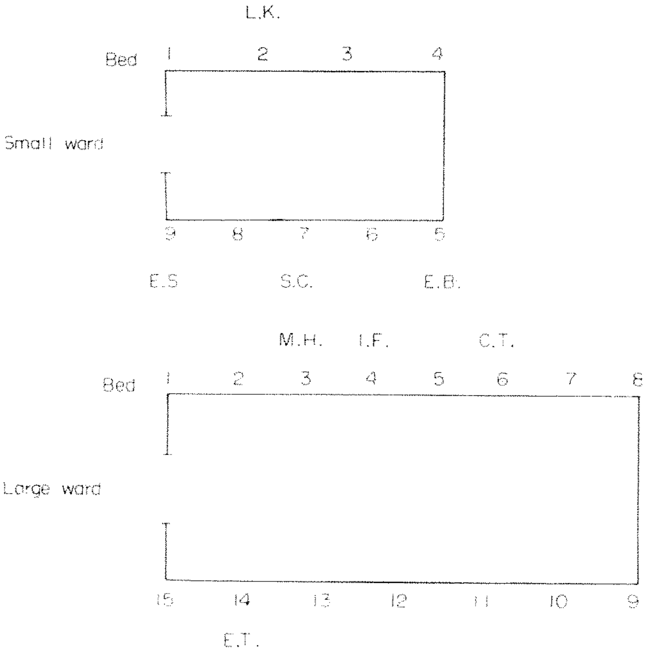

FIGURE 1. Diagram of lay-out of Ward, A, showing bed positions of affected patients.

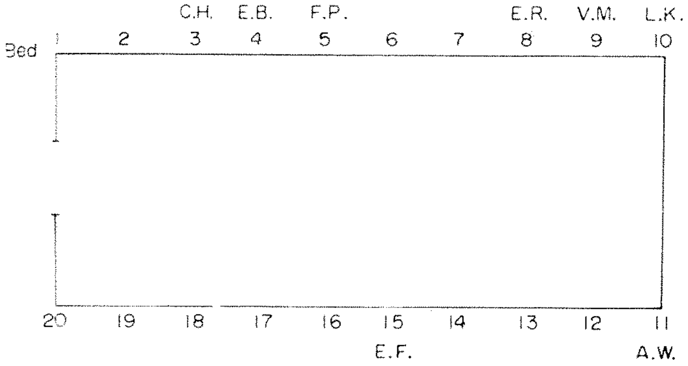

FIGURE 2. Diagram of lay-out of Ward B, showing bed positions of affected patients.

they became soiled; blankets were washed as necessary. Foam rubber rings with either cotton or polythene covers were used to relieve the pressure areas of some patients. Zinc cream and other bland ointments were used by the nursing staff to massage the backs of some bed-ridden patients. These creams were supplied direct from the pharmacy in clean jars about once a week. Bed pans were sterilized by machine, using hot water, after use, and the seats of commodes in the wards were swabbed with Stericol after use by each patient. The commodes had webbing straps to support the patients. A plastic covered hoist was used to lift three or four obese patients from both wards into the bath. The main surface of this hoist was shiny and clean, but considerable quantities of debris were lodged under the plastic that bound its edges.

Skin scrapings for mycological examination were collected from clinically affected areas of the patients' skin, and submitted to the laboratory. The feet and toe nails of all affected patients were examined clinically, and scrapings and/or toe nail clippings were taken. All unaffected patients in the two wards were examined clinically, particular attention being paid to their feet. Skin scrapings and toe nail clippings were taken from any patients who were suspected of having tinea pedis.

Skin scrapings and nail clippings were examined microscopically in KOH squashes, and cultured on glucose–peptone agar with added chloramphenicol and actidione. This medium was also used in all attempts to culture fungi from patients' fomites and objects in use in the wards, as described below. Blankets were sampled by means of a new vacuum cleaner with a disposable dust-collecting bag, the bag being changed for each blanket. In the laboratory each bag was cut open, and the contents spread on twelve petri dishes. The foam rubber rings and their covers were pressed directly on to the surface of the agar in the petri dishes, using about six dishes for each object. Webbing straps from the commodes were scraped over open petri dishes. The shiny surface of the bath hoist was swabbed, and the swabs plated. The accumulated debris under the bindings of the hoist was scraped out and plated. Dust, collected on dry swabs from the tops of ward doors and other similar places, was plated in the normal way.

CLINICAL OBSERVATIONS

In August 1972, a 75-year-old incontinent female patient (M.H.) from Ward A was referred for a dermatological opinion. She had had patches of erythematous skin with peripheral scaling on her back for 2 months. There was no evidence of skin disease elsewhere. Direct microscopy of skin scrapings showed fungus to be present, and *T. rubrum* grew on culture. The patient was treated with local tolnaftate cream and systemic griseofulvin 0·5 g daily, and her rash cleared.

In October 1972, an 88-year-old incontinent patient (A.W.) was seen in Ward B with similar skin lesions on her buttocks and beneath her breasts. *T. rubrum* was isolated. At the same time, an 82-year-old woman (L.K.) in Ward B was referred with a crusted and pustular area of alopecia affecting the right frontoparietal region of the scalp (Fig. 3). This lesion had apparently first appeared 2 months earlier, when the patient was on Ward A, and had been present when she was transferred to Ward B in September 1972. Bacterial cultures from the skin lesion grew *Staphylococcus aureus*, and the patient was treated with both systemic and local antibiotics. The area of crusting did not improve, but gradually increased in size, and specimens of hair were eventually taken for mycological examination. Microscopy showed an ecto-endothrix infection of the hairs, and *T. rubrum* was grown on culture; treatment with griseofulvin and local benzoic acid compound ointment was commenced in November 1972.

During the period October 1972 to February 1973, eleven other elderly female patients in Wards A and B developed *T. rubrum* infections, diagnosed in all cases by culture of the fungus from skin scrapings (Tables 1 and 2). In all except one of these patients (C.T.), the main sites of skin involvement were the buttocks, the backs of the thighs, or the sacral and lumbar regions (Fig. 4). In one patient (C.T.) the patches occurred on the lower abdomen, but not on the buttocks or back. The involved areas of skin were a uniform red or brown colour, with very slight scaling, and had a sharp junction with adjacent normal skin. There was often a certain amount of loose and overhanging scale at the periphery of the lesion. All patients were treated with local tolnaftate cream and systemic griseofulvin. The fungous infection cleared in all patients, but in two (E.S. and E.F.) it recurred after cessation of therapy. In both these cases treatment was recommenced using a higher dose of griseofulvin (1 g daily).

With reference to the apparent particular susceptibility of the backs of the thighs, the buttocks, and the lower back to infection, it was noted that twelve of the fourteen affected patients were incontinent of urine, and seven were also incontinent of faeces. One of the two patients who were not incontinent

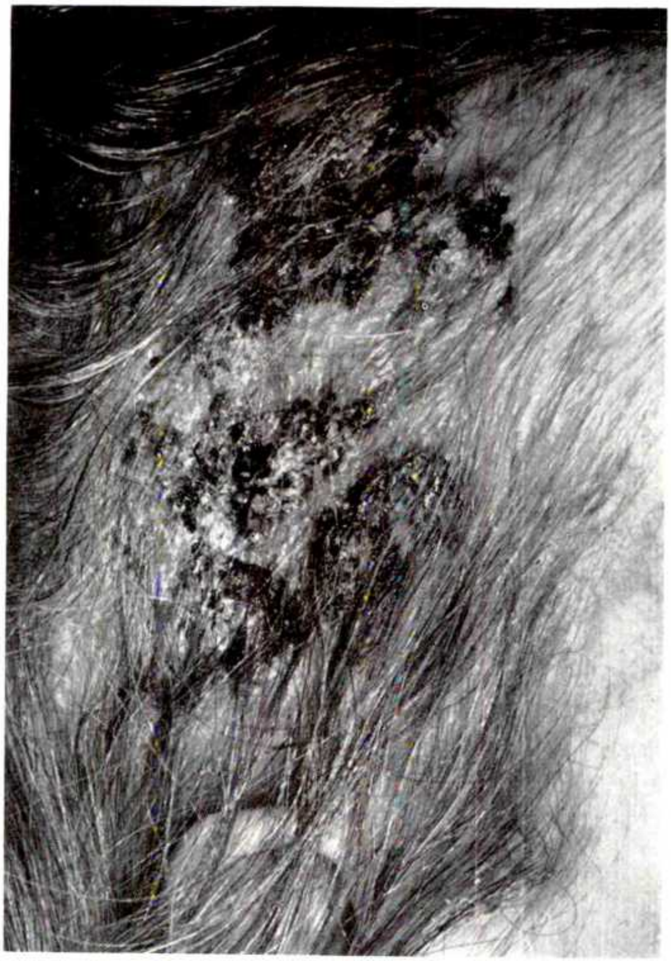

FIGURE 3. Area of partial alopecia with crusting and pustulation on parietal region of scalp (patient L.K.).

was L.K., whose infection was confined to the scalp. Table 3 shows the relationship of infection to continence and incontinence in the two affected wards.

MYCOLOGY

The same, somewhat unusual strain of *T. rubrum* was isolated from all the patients except one (F.P.). The strain was a very deep wine-red in colour, and the aerial mycelium was confined to a small area at the centre of the colony. The mycelium bore very large numbers of typical macro- and microconidia, and for this reason the strain must be considered an atypical 'granular' type of *T. rubrum* (Bang, 1910).

The strain isolated from patient F.P. differed from all the other isolates in having a thick white 'duvet' of aerial mycelium, reminiscent macroscopically of the 'downy' strain of *T. rubrum* (Castellani, 1910). Unlike that strain, however, F.P.'s isolate had large quantities of macro- and microconidia, identical with those of the other isolates in the outbreak. It seemed likely that it was a mutant from

TABLE 1. Details of affected patients in Ward A

Patient	Age (years)	Date admitted	Onset of rash	Site	Whether incontinent
M.H.	75	Nov. 1969 (intermittent)	June 1972	Back	Double incontinence
L.K.	82	1960 Transferred to Ward B 25.9.72	Aug. 1972	Scalp	No
E.S.	77	11.9.72	Noted 20.10.72	Back Back of thighs Back of legs	Double incontinence
E.B.	62	28.3.72 Transferred to Ward B 27.10.72	Noted 27.10.72	Buttocks	Double incontinence
I.F.	58	Feb. 1969 (intermittent)	Noted 13.1.73	Buttocks	Double incontinence
C.T.	84	2.9.72	Noted 20.1.73	Lower abdomen	Urine (occasionally)
S.C.	85	12.5.69	Noted 25.1.73	Buttocks	Double incontinence
E.T.	78	23.11.72	Noted 7.2.73	Buttocks Sacral area	No

TABLE 2. Details of affected patients in Ward B

Patient	Age (years)	Date admitted	Onset of rash	Site	Whether incontinent
L.K.	82	Transferred from Ward B 25.9.72	Aug. 1972	Scalp	No
A.W.	88	25.9.72	Present when seen 9.10.72	Below breasts Buttocks	Double incontinence
C.H.	82	20.9.72	Noted 12.10.72	Back Buttocks	Double incontinence
E.B.	62	Transferred from Ward B 27.10.72	Present on arrival 27.10.72	Buttocks	Double incontinence
E.R.	88	28.9.72	Noted 2.11.72	Back Buttocks	Urine
F.P.	82	25.9.72	Noted 2.11.72	Buttocks	Urine
V.M.	89	2.10.72	Noted 5.11.72	Sacral area Buttocks	Urine
E.F.	82	13.10.72	Noted 8.11.72	Buttocks	Urine

the predominant strain. The instability of F.P.'s isolate was confirmed when a colony on the original isolation plate produced a further mutant, with faster growth than the parent strain, and whose aerial mycelium was sterile except for a few microconidia.

All the isolates of *T. rubrum* from the inanimate objects discussed below were of the atypical 'granular' strain found on thirteen of the fourteen patients.

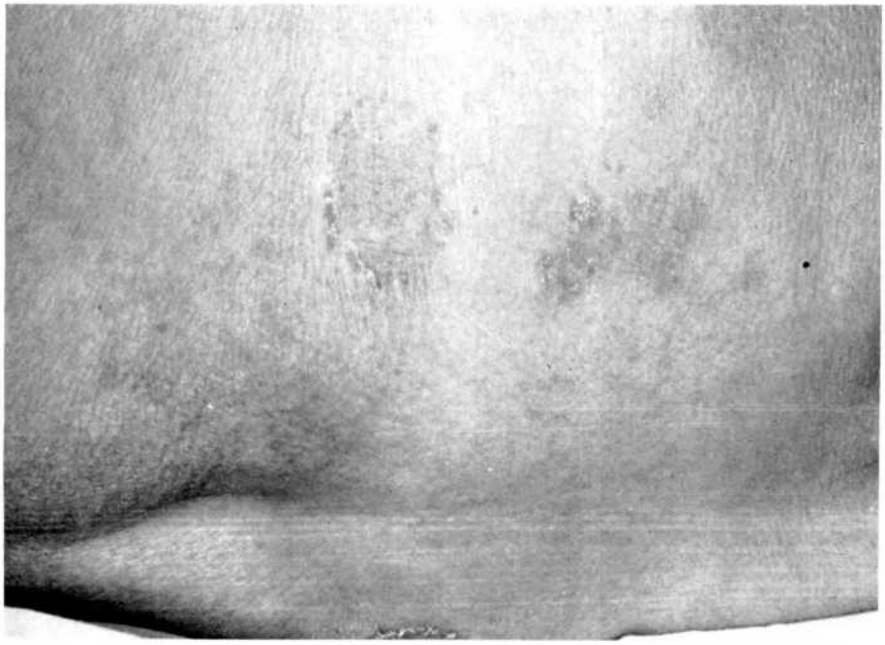

FIGURE 4. Typical patches of *T. rubrum* infection on lumbar region of incontinent patient.

TABLE 3. The relationships of infection to incontinence in Wards A and B

Patients	Continent	Incontinent
Ward A (24 patients)		
Affected patients	2	6
Unaffected patients	9	7
Ward B (20 patients)		
Affected patients	1	7
Unaffected patients	8	4

SPREAD OF INFECTION

It is uncertain how this epidemic originated. None of the patients in either ward had *T. rubrum* infection of the feet or toe nails. M.H. who, for 5 years, had been admitted intermittently to Ward A for 6 weeks in every 3 months, first developed her skin lesions in June 1972, but had not, apparently, been in contact with *T. rubrum* at home. Her husband had no clinical evidence of disease. Patient

L.K. was at this time on Ward A, and developed the area of crusting on her scalp about August 1972. This was still undiagnosed when she was transferred to Ward B in September 1972, and treatment was not started until she had been in Ward B for a month.

Attempts were made to trace the way in which infection spread. In Ward A, four patients out of a total of fifteen were affected in the larger ward, and four out of nine in the smaller ward. In Ward B, six of the eight affected patients were on one side of the ward (patients L.K. and E.B. were both transferred from Ward A to this side of Ward B). Blankets from one unaffected and seven infected patients were sampled for fungus, but in each case after the patient had been cured or was already receiving treatment. Thirty colonies of *T. rubrum* were obtained from L.K.'s blanket, but the other blankets yielded only one colony of the fungus between them (from E.B.'s blanket). Specimens of dust and fluff from both wards, taken after most patients had been successfully treated, did not grow fungus on culture. The fungus was not isolated from one cotton cushion cover sampled, but twenty-one colonies were recovered from the polythene cover of the foam rubber ring used by patient E.T. while her buttocks were actively infected. The pillow-case of L.K. was not sampled until she had been under treatment for some weeks, and no fungus was found. Attempts to culture from the webbing commode straps, and the plastic covered bath hoist, were also unsuccessful: in the case of the bath hoist this may have been due to the extensive overgrowth of all culture plates by Proteus.

Nursing care to the backs of incontinent patients on the two wards usually involved regular turning of the patient, washing with soap and water, and powdering with the patients' own talcum powder. In three or four cases, zinc cream was regularly applied to the back. Specimens of creams from used jars, taken from the surface where the nurses' finger marks were clearly visible, were cultured for fungi, but none was found.

The unusual distribution of the patches of ringworm in this outbreak, involving particularly the backs of the thighs, the buttocks and the lower back, suggested that in some way *T. rubrum* was being spread particularly to these areas. The epidemiological investigation described above seemed to eliminate the likelihood of indirect transmission to these particular sites, but the possibility of carriage of infection by the nurses' hands, when turning patients or during treatment of the skin of the patients' backs, had to be considered. The hands of all nursing staff on the two wards were examined, but no clinical evidence of fungal infection was found.

DISCUSSION

The outbreak of *T. rubrum* infection reported here was unusual in that the lesions in almost all the affected patients were sited over the buttocks, the backs of the thighs, and lumbosacral region. No patients showed involvement of the feet or nails. The isolation of the same distinctive strain of the fungus from all patients but one, and from all fomites, is good evidence that this was indeed an outbreak originating from a single source, and not a series of new infections.

The extremely high incidence of incontinence in the affected patients, compared with unaffected patients in the same wards, suggests that this factor was probably of major importance in predisposing certain patients to infection, and in determining the site of skin involvement. In spite of painstaking nursing care, most of the affected patients, many of them demented, were often to be found lying in wet and soiled beds.

Attempts to trace the way in which infection was transmitted from case to case were not successful. Patient L.K., who had a scalp lesion but no evidence of infection elsewhere, was a common link between the two wards, and it is thought that dissemination of the fungus to other patients occurred during the 3 months that her scalp condition remained undiagnosed. Not only would fungal particles from a scalp lesion be much more readily subject to air dispersal than those from a covered body lesion, but the very numerous arthrospores in infected hairs could be dispersed individually, and much more easily than mycelium embedded in fragments of skin. This may account for the heavy contamination of L.K.'s blanket, and the fungus could well have been further dispersed during bed making. It can be seen that a small group of affected patients were sited near to the bed of L.K., but this grouping may have been due to chance.

We were unable to find any evidence of direct transmission of infection via fomites, and there was no evidence of skin disease affecting the hands of any nurses on the two wards. It is, however, possible that staff hand-washing was neglected on isolated occasions, and that transmission of fungus from one patient to another occurred on the hands of medical or nursing staff. If the fungus was transmitted in this way, the damp, warm and possibly abraded skin on the backs of incontinent patients would be a suitable and likely site for successful colonization.

A similar outbreak of *T. rubrum* infection in a geriatric hospital in South Australia was reported by Hakendorf in 1952. Eighteen patients were affected, and in most cases the lesions commenced either on the buttocks or the abdomen. The infection was thought to have originated from a patient who had widespread but undiagnosed lesions due to *T. rubrum*, and it was supposed that spread of infection had occurred by the use of inadequately sterilized bed pans, or the hands of the nursing staff. No information was given in this paper as to whether the affected patients were incontinent.

Reports of *T. rubrum* affecting scalp hair are extremely rare in the European and American literature, but when this occurs the clinical picture is variable (Price, Rosenthal & Villafane, 1963). Cases have been reported showing granulomata, kerions, and black dot alopecia. The infection may be either ectothrix or endothrix. The case reported here showed an extending area of alopecia with scaling and erythema, areas of crusting and pustulation, and ecto-endothrix infection of the hairs. It is of some interest to note that Desai & Bhat (1961), in India, found five cases of scalp ringworm in a total of 364 patients infected with *T. rubrum*, a very high incidence when compared with the rarity of scalp infection due to *T. rubrum* in Europe.

The part played by various factors in the spread of *T. rubrum* of the feet is still debated. Baer *et al.* (1955) concluded that infection occurred as a result of diminished host resistance, while Gentles & Holmes (1957) produced evidence to show that although personal immunity was important, exposure to infection was often of even greater moment. English (1957) showed that spread of *T. rubrum* was not uncommon within the families of affected subjects, and that infection usually started on the feet. The minimal duration of exposure before clinical infection occurred was 1 year.

While the primary site of *T. rubrum* infection in Europe is usually the feet, in India this is rare, and primary infections usually occur on the trunk, involving especially the waist-line, crural region, and other areas of the glabrous skin (Desai, 1966). These differences have been attributed to differences in dress habits, particularly the fact that Indian clothing causes friction at the waist, and Europeans keep their feet continually covered. Climatic factors, and wide differences in the standard of living, are undoubtedly also important in the different epidemicity of *T. rubrum* in Europe and India. One other factor of possible importance is that the strain of *T. rubrum* predominating in Europe, the 'downy' strain (Castellani, 1910), is distinct from that found in India, the 'granular' strain of Bang (1910). In the present epidemic, the strain of *T. rubrum* that was isolated closely resembled the 'granular' type of the fungus, and it is possible that this strain is better adapted to growth on glabrous skin than is the 'downy' type. It should, however, be noted that in the Australian outbreak reported by Hakendorf (1952), the fungus isolated was the usual 'downy' strain (Donald, 1973).

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Reactive perforating collagenosis

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SUMMARY

Five cases of reactive perforating collagenosis (RPC) are reported. Four of these cases were in a father and his three children. This is the first reported instance of RPC occurring in more than one generation.

The clinical and histological features of the disease are described and compared with other disorders showing perforating skin lesions, such as Kyrle's disease, perforating folliculitis and elastosis perforans serpiginosa.

Evidence is presented to show that RPC is a genetically determined disorder with an inherited predisposition to an unusual skin reaction to superficial trauma.

Reactive perforating collagenosis (RPC) has been delineated as a distinct clinical and pathological entity (Mehregan, Schwartz & Livingood, 1967). The disease usually starts in childhood as small papules, which become umbilicated with a keratinous plug. The lesions regress spontaneously in about 6–8 weeks, but new lesions appear as older ones involute. It has a distinct histological pattern characterized by necrobiosis of superficial collagen, which becomes haematoxylinophilic. The collagen changes are produced by an abnormal reaction of the skin to superficial trauma. The altered collagen is eventually extruded through the epidermis by a peculiar mechanism known as 'epithelial elimination' (Mehregan, 1968). Weiner (1970) noted that information concerning the disease was confined to about twelve cases and reported two additional cases of his own. In 1971, Pasricha, Girgla & Kandhari reported a case from India.

We have been able to study five cases of RPC, four of which occurred in a father and his three children. Apart from the rarity of the condition, this seems to be the first instance of the disease occurring in two generations. The purpose of this paper is to describe the clinical, pathological and genetic features of this condition and compare it with other diseases showing perforating lesions of the skin, with which RPC has been confused in the past.

CASE REPORTS

Case 1

A male patient aged 51 years was seen in March 1973 with multiple, asymmetric papules all over the body. The lesions showed a predilection for extensor aspects of the extremities. The disease started at the age of 10 years. Early lesions were small skin coloured papules. As they enlarged they developed a central depression filled with a keratinous plug. The keratinous plugs were adherent and their removal caused bleeding. The lesions gradually enlarged and some of them reached a maximum size of about 8 mm in a month. The keratinous plugs also enlarged, becoming hard and leathery (Fig. 1). The Köbner phenomenon was present. The lesions regressed spontaneously in 6–8 weeks. The papules flattened, the keratinous plugs were cast off and healing occurred with slight atrophy and mild pigmentary changes. Some of the lesions became inflamed and pustular with secondary infection.

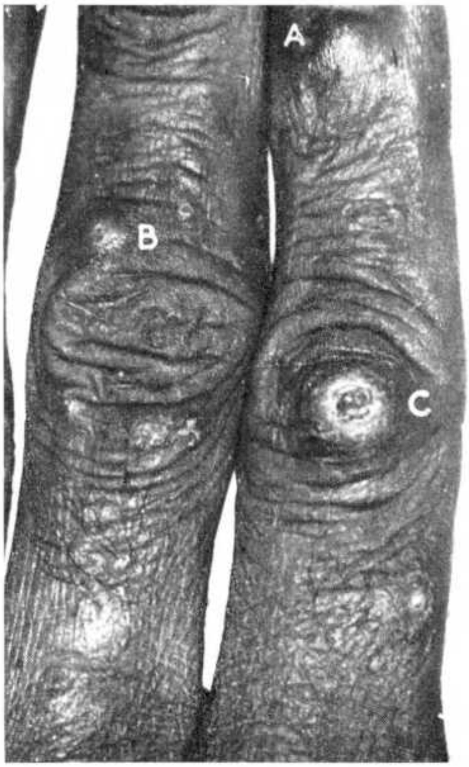

FIGURE 1. Several lesions on dorsa of fingers: an early skin coloured papule (A); a more advanced lesion becoming umbilicated (B); large umbilicated lesion in the early stage of regression (C).

The general health of the patient was good. There was no evidence of any hepatic or renal damage or any other systemic disease. Laboratory investigations showed no abnormality, other than a glucose tolerance test indicative of mild diabetes mellitus.

He was married, but there was no similar disease in his four sons, four daughters, or other members of his family. His parents were not consanguineous.

A biopsy of a well developed papule from the forearm showed a cup-shaped depression of the epidermis filled with a plug, made up of a mixture of ortho- and parakeratotic material, necrobiotic collagen and degenerating nuclei of inflammatory cells. The wall of the crater was oedematous and acanthotic, and adjacent areas of the epidermis showed prominence of the granular layer. At the lower border of the depression the epidermis was thinned and disrupted in places, through which necrobiotic collagen fibres could be seen extruding into the keratinous plug (Fig. 2). Much of the collagen in the superficial layers of the dermis showed haematoxylinophilic rather than the normal eosinophilic staining. The perforating collagenous bundles were better demonstrated by van Gieson's stain. Verhoeff's stain showed no changes in the appearance or quantity of elastic tissue and no elastic tissue was found in the keratotic plug.

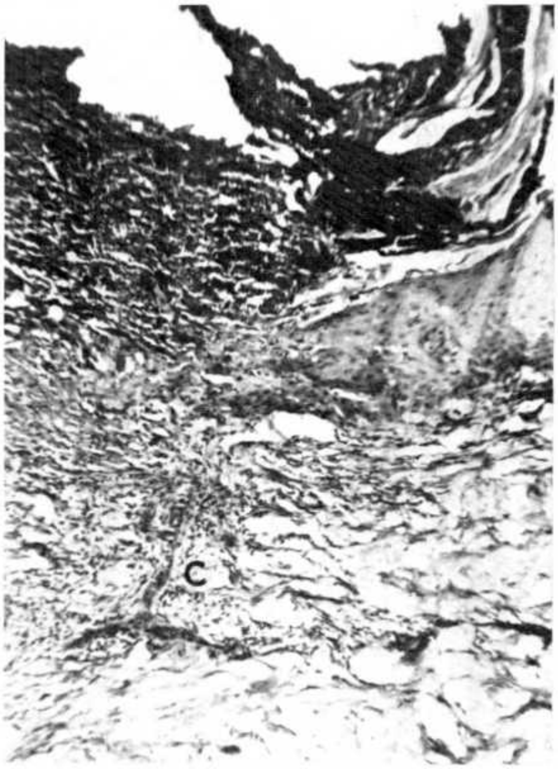

FIGURE 2. Well developed umbilicated lesion showing cup-shaped depression of the epidermis, containing a large plug consisting of a mixture of ortho- and parakeratotic keratin, necrobiotic connective tissue and degenerating nuclei of inflammatory cells. A vertically arranged collagen bundle (C) is seen extruding through the disrupted base of the epidermal crater. (van Gieson, × 100).

Case 2

A male aged 40 years was seen in August 1973. His disease started at the age of 3 years. The morphology and evolution of the lesions were similar to case 1, but he had more extensive lesions all over the body, including the face, scalp, palms and soles. More lesions were found on the dorsa of the hands and extensor aspects of the extremities. There were no mucosal lesions.

Biopsy examination showed a picture essentially similar to that seen in case 1. Laboratory investigations showed no abnormalities. His glucose tolerance test was normal.

He had married his paternal sister's daughter. His wife had no clinical evidence of the disease, but his wife's sister had lesions similar to those of the patient. Three out of his four children also had the disease (Fig. 3). His first daughter (case 3), aged 12, had a few lesions on the extensor aspects of the extremities and trunk. The lesions started at the age of 3 years. The second daughter (case 4), aged 10,

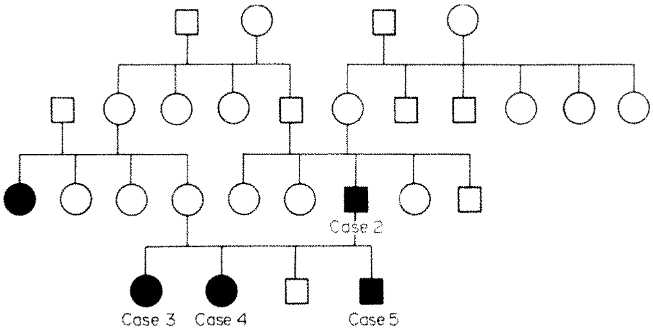

FIGURE 3. The family pedigree showing two generations of affected members. ●, affected female; ○, unaffected female; ■, affected male; □, unaffected male.

had very few lesions, which appeared at the age of 5 years. The third child, a boy of 6, has no clinical evidence of the disease. The fourth child (case 5), a boy of 1½, has already developed a few lesions on the extremities.

Apart from clearing of the secondary pyoderma, antibacterial therapy, vitamin A 100,000 units i.m. daily for 30 days and local keratolytic ointments did not make any appreciable alteration in the natural history of the disease.

DISCUSSION

All the five cases reported here showed clinical and histological features of reactive perforating collagenosis as described by Mehregan *et al.* (1967). However, the initial diagnosis in the first case was Kyrle's disease. This is a common mistake. Many cases diagnosed as Kyrle's disease in the past have been shown to be some other condition with perforating lesions of the skin such as perforating folliculitis, reactive perforating collagenosis or elastosis perforans serpiginosa. All these conditions have the common feature of keratotic papular lesions. However, the total picture is usually sufficiently distinctive to allow differentiation on clinical grounds alone.

In Kyrle's disease, which occurs mainly in adults, the lesions are follicular or parafollicular brownish red keratotic papules, which tend to aggregate into plaques. The lesions are usually bilateral and tend to favour the lower extremities. The disease is known to be associated with diabetes mellitus, hepatic and renal disease (Carter & Constantine, 1968). The presence of diabetes mellitus in the first case in this series of RPC seems to be the first reported instance of this association. The significance of this can be assessed only by further studies.

Perforating folliculitis is a relatively common disease that affects all ages. The lesions are discrete erythematous follicular papules, with a central keratotic plug containing a curled-up hair. There is a predilection for the extensor aspects of the upper arms, buttocks and upper thighs, but no Köbner phenomenon (Mehregan & Coskey, 1968).

Elastosis perforans serpiginosa occurs mainly in young adults. The lesions are few and usually localized to one site on the neck, extremities or trunk. They show an annular or serpiginous configuration and no Köbner phenomenon (Mehregan, 1968).

In spite of the fairly distinctive clinical features of these diseases with perforating lesions of the skin, sometimes a final diagnosis can be arrived at only on histological examination. All these diseases show perforation of the epidermis or follicular wall. In Kyrle's disease the perforation occurs from without inwards. The keratotic plugs penetrate through the epidermis causing a granulomatous inflammatory reaction in the subjacent dermis. Mild degenerative changes are seen in the connective tissue, but there is no alteration in the elastic tissue.

In RPC, perforating folliculitis and elastosis perforans serpiginosa there is a peculiar phenomenon called 'transepithelial elimination', in which a series of epithelial changes in the skin lead to elimination of foreign material from the corium through the epidermis or follicular wall, without producing major disturbances in these structures (Mehregan, 1968). In elastosis perforans serpiginosa the hyperplastic and thickened elastic fibres act as an irritant foreign material. In perforating folliculitis the keratinous plug, with the retained hair inside, causes mechanical irritation and produces perforation of the hair follicle. This is followed by elimination of connective tissue and elastic fibres through the area of follicular perforation.

In RPC, as an abnormal response to superficial trauma, the collagen in the papillary layer of the corium becomes altered and haematoxylinophilic. This altered collagen causes irritation and perforation of the epidermis with 'transepidermal elimination' of the necrobiotic connective tissue. This hypothesis of the pathogenesis of RPC, first put forward by Mehregan *et al.* (1967), is supported by the following findings. The Köbner phenomenon has occurred in almost all the cases reported; in one case reported by Weiner (1970) lesions developed following corporal punishment. Bovenmyer (1970) noted lesions following insect bites and acne vulgaris and successfully produced lesions by needle scratch.

Our cases and those reported by other workers (Mehregan *et al.*, 1967; Weiner, 1970) suggest a genetic origin of the disease. The onset of the disease in childhood, the occurrence in more than one generation, and the apparent rarity support this. We feel that RPC is a genetically determined disorder with an inherited predisposition to an unusual skin reaction to superficial trauma.

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Familial reactive perforating collagenosis and intolerance to cold

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SUMMARY

A clinico-pathological study of reactive perforating collagenosis (RPC) is reported in seven adult siblings of both sexes in two unrelated consanguineous families from Kuwait. Although the primary defect in the skin of these patients is genetically determined, superficial trauma seems to be a triggering factor in most of the lesions. However, the regular occurrence of spontaneous lesions on the face in two patients and perniosis of the hands and feet in four other patients, together with the increased incidence on the exposed areas during the cold season, suggest an additive role for atmospheric cold. Experimental reproduction of RPC lesions was successful on the dorsa of the wrist and right middle finger respectively in two patients, while it failed twice on the covered areas. A significant finding was the absence of such lesions following deep incisional wounds, which suggests that the primary defect is in the pars papillaris of the dermis where a post-traumatic necrobiotic change starts off an RPC lesion.

Mehregan, Schwartz & Livingood, in 1967, reported the first case of reactive perforating collagenosis (RPC), a curious reaction of the skin to superficial trauma, in a 6½-year-old girl. Later, Mehregan (1970) reviewed the findings in ten other cases which had come to his attention. Bovenmyer (1970) reported another case and succeeded in producing an experimental linear lesion in his patient, and Weiner (1970) added two more cases in siblings. In this communication seven patients with RPC in two unrelated consanguineous families from Kuwait are reported.

CASE REPORTS

Family A: Case 1 (*B.M.*)

The propositus in this family, a 26-year-old dark Kuwaiti male, attended the skin clinic, Amiri Hospital, in October 1967, for a symptomless, recurrent eruption of spontaneously-healing, umbilicated papules on his face, hands and feet which had been present since the early years of his childhood. Single lesions usually followed a scratch or an abrasion, but those on his face seemed to occur spontaneously during the cold season each year.

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Physical examination, except for the skin lesions, proved noncontributory. The skin lesions on his face consisted of several raised, acne-like, maculopapular spots of 2–4 mm diameter on the forehead, sides of the face and chin.

On his first visit some of these lesions had healed leaving hyperpigmented depressed spots, others were in the healing stage, while several umbilicated lesions were still active (Fig. 1). A fully developed single lesion of 4 weeks duration on the dorsum of his right middle finger consisted of an umbilicated skin-coloured firm papule 1 cm in diameter. Another resolving lesion was noted on the dorsum of the terminal phalanx of his right index finger.

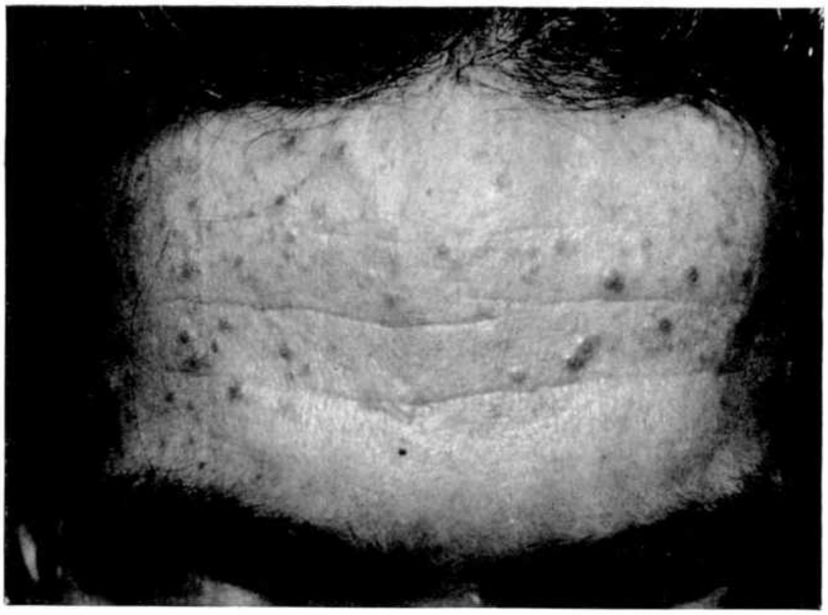

FIGURE 1. Spontaneously erupting RPC lesions on the forehead (case 1) in various stages during the cold season.

Subsequent examinations at short intervals revealed gradual regression of the lesion on the right middle finger with complete resolution within 8 weeks to leave a bluish macule which took a few more months to vanish. In December 1967, the patient volunteered to produce an experimental lesion. After electric shaving and cleaning with 70% alcohol, a rectangular area of skin 15 × 8 mm over the lower end of the ulna on the back of his left wrist was abraded by linear scarification just adequate to cause visible bleeding with the sharp edge of a scalpel blade. A raised, crusted plaque erupted within 1 week and developed into an umbilicated, firm papule 15 × 8 mm. This subsequently underwent changes identical to those in non-experimental lesions, taking almost 8 weeks to resolve and leave a hyperpigmented, depressed spot but no scar.

On another occasion a superficial scalpel incision deep enough to produce visible bleeding on the medial aspect of the right scapula failed to produce an RPC lesion.

It was remarkable that an emollient cream (Oilatum) rubbed into his face daily during the cold winter months could almost prevent the eruption of spontaneous lesions.

The clinical findings in cases 2 and 3 in this family are summarized in Table 1.

Investigations. Routine laboratory blood tests, serum protein electrophoresis, lipid profile, urine

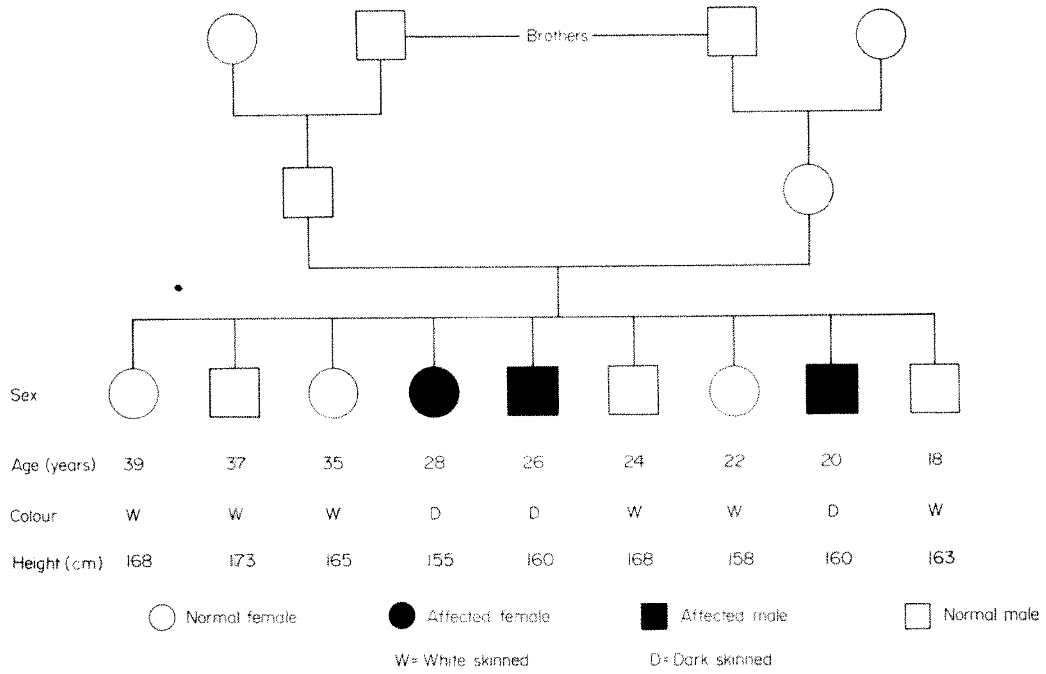

FIGURE 2. Family A: family tree showing affected members and the relevant data.

tests and stool examinations were within normal limits. Chromosomal studies performed by Dr O. Elfi (geneticist at Sabah Hospital) on the two affected brothers showed normal karyotypes.

Histopathology. Biopsies from well-developed lesions in the two affected brothers were stained with Haematoxylin–eosin, acid orcein–Giemsa, Van Gieson, Alcian blue–PAS, toluidine blue and aldehyde fuchsin methods. Our findings were similar to those described by Mehregan *et al.* (1967) in their first case of RPC. Dr Amir Mehregan of Michigan, who kindly examined the paraffin block prepared from a 4-week-old lesion on the dorsum of the right middle finger of the propositus, reported the following (Fig. 3).

'The sections show a central area of complete loss of the epidermis forming a cup-shaped area of depression containing a solid mass of necrotic crust. The crust is made up of pieces of necrotic connective tissue, numerous degenerating inflammatory cells and keratinous flakes. There are also several vertical thin collagen bundles which stain pinkish-red in Van Gieson sections. The acid orcein–Giemsa and aldehyde fuchsin stains show no elastic fibres within the crust. The epidermis surrounding this area is moderately acanthotic. The upper corium shows a few slightly dilated capillary blood vessels surrounded by a mild infiltrate of lymphocytes, polymorphs (neutrophils) and a few eosinophils. There is no increase in the number of elastic fibres in the corium. There is also no deposition of acid mucopolysaccharides in sections stained by Alcian blue–PAS, aldehyde fuchsin or toluidine blue. The changes are typical of a well-developed umbilicated lesion of reactive perforating collagenosis.'

Another biopsy of an acne-like lesion from the forehead of the same patient showed an RPC lesion in the resolving stage. The central pit exhibited partial closure across its bottom by regenerating epithelium with hyperplasia of the stratum granulosum around the upper part of the pit.

Family B: Case 4 (*A.G.*)

The key case in this family was a 40-year-old fair, white Palestinian Arab living in Kuwait for the last

TABLE 1. Reactive perforating collagenosis: important clinical data

Case no.	Author		Sex	Age of onset	Age of presentation (years)	Type of trauma
1	Kanan	Case 1: dark and short	M	Early childhood	26	Scratches and abrasions
2	Kanan	Case 2: dark and short	F	Early childhood	28	Scratches and abrasions
3	Kanan	Case 3: dark and short	M	Early childhood	20	Scratches and abrasions
4	Kanan	Case 4: fair	M	Early childhood	40	Scratches and abrasions
5	Kanan	Case 5: fair	M	Early childhood	32	Scratches and abrasions
6	Kanan	Case 6: fair	M	Early childhood	32	Scratches and abrasions
7	Kanan	Case 7: fair	F	Early childhood	24	Scratches, abrasions and mosquito bites
8	Mehregan et al. (1967)		F	9 months	6½	Superficial scratches
9	J. Weiner (1967)	Siblings	F	Early infancy	11	Superficial scratches
10	J. Weiner (1967)		M	Early infancy	6	Superficial scratches
11	Mehregan (1970)		Not stated	Adult	Adult	Superficial scratches
12	Mehregan (1970)		Not stated	Childhood	Adult	Superficial scratches
13	Mehregan (1970)		Not stated	Adult	Adult	Superficial scratches
14	Mehregan (1970)	Siblings	Not stated	Childhood	Childhood (5–14 years)	Scratches and mosquito bites
15	Mehregan (1970)		Not stated	Childhood	Childhood (5–14 years)	Scratches and mosquito bites
16	Mehregan (1970)		Not stated	Childhood	Childhood (5–14 years)	Scratches and mosquito bites
17	Mehregan (1970)	Siblings	Not stated	Childhood	Childhood (5–14 years)	Scratches and mosquito bites
18	Mehregan (1970)		Not stated	Childhood	Childhood (5–14 years)	Scratches and mosquito bites
19	Bovenmyer (1970)		F	4 years	6	Scratches
20	A. Weiner (1970)		M	6 years	6	Not constantly obtained
21	A. Weiner (1970)		F	18 months	8	Sites of corporal punishment on one occasion

Affected sites	Unaffected areas	Parental consanguinity	Other findings
Face, hands (dorsa) and feet	Trunk, palms and soles	Positive	Spontaneous lesions on face in cold season
Face, hands and feet (dorsa)	Trunk, palms and soles	Positive	Spontaneous lesions on face in cold season
Hands and feet	Trunk, palms and soles	Positive	—
Hands and feet	Trunk, palms and soles	Positive	Intolerance to cold (chilblains on hands and feet)
Hands and feet	Trunk, palms and soles	Positive	Intolerance to cold (chilblains on hands and feet)
Hands and feet	Trunk, palms and soles	Positive	Intolerance to cold (chilblains on hands and feet)
Hands and legs	Trunk, palms and soles	Positive	Intolerance to cold (chilblains on hands and feet)
Dorsa of hands, forearms, elbows and knees	Trunk, palms and soles	? negative (not stated)	Rickets-like bone changes
Scalp, hands (dorsa), forearms (extensor), arms and legs	Trunk, palms and soles	? negative (not stated)	—
Scalp, hands (dorsa), forearms (extensor), arms, legs and face	Trunk, palms and soles	? negative (not stated)	—
Scalp, hands (dorsa), forearms (extensor), arms, legs and trunk	Palms and soles	? negative (not stated)	—
Scalp, hands (dorsa), forearms (extensor), arms, legs and trunk	Palms and soles	? negative (not stated)	—
Scalp, hands (dorsa), forearms (extensor), arms, legs and trunk	Palms and soles	? negative (not stated)	—
Hands (dorsa), arms and legs	Trunk, palms and soles	? negative (not stated)	—
Hands (dorsa), arms and legs	Trunk, palms and soles	? negative (not stated)	—
Hands (dorsa), arms and legs	Trunk, palms and soles	? negative (not stated)	—
Hands (dorsa), arms and legs	Trunk, palms and soles	? negative (not stated)	—
Hands (dorsa), arms and legs	Trunk, palms and soles	? negative (not stated)	—
Face, extremities and buttocks	Palms, soles and upper thighs	Negative	Spontaneous RPC lesions in winter at acne sites on face and minimal itching
Face, extremities and trunk	Palms and soles	? negative	Lesions pruritic
Face, extremities and trunk	Palms and soles	? negative	Lesions pruritic

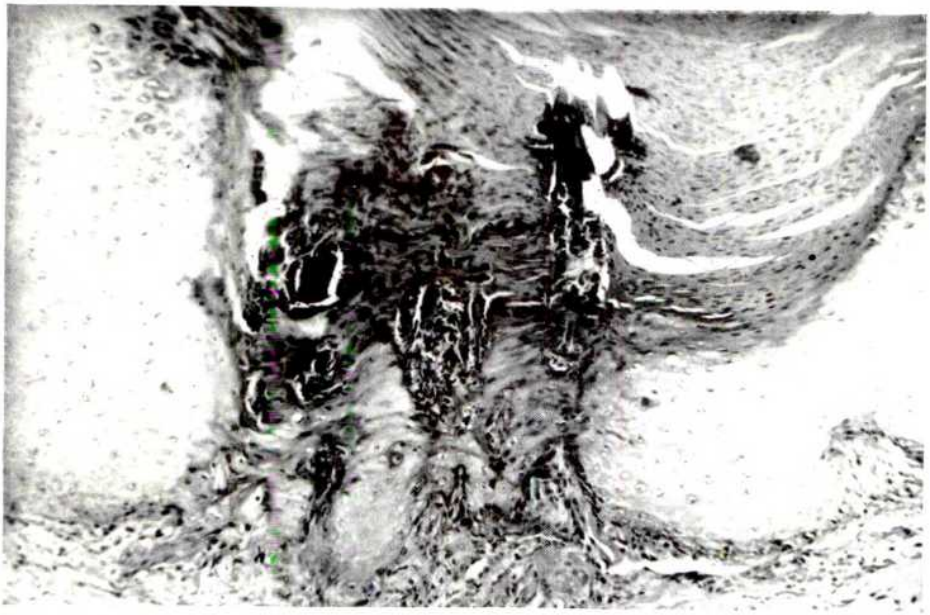

FIGURE 3. A cup-shaped RPC lesion showing perpendicularly orientated collagen strands being extruded among the debris of the central keratinous plug (Van Gieson stain, × 50).

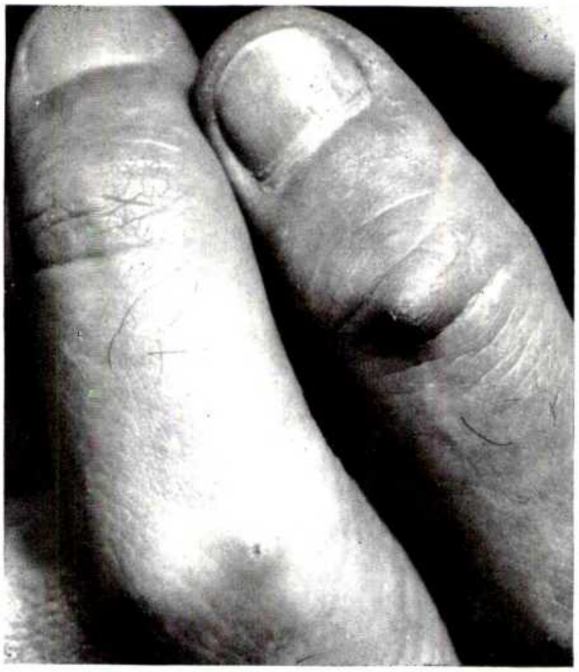

FIGURE 4. Mature RPC lesions (5–6 weeks) on the dorsa of both thumbs in a white male patient (case 4).

20 years and working as a technician in the department of general pathology, Sabah Hospital, Kuwait. By chance, he was noticed by the author on 27 October 1971 to have two dome-shaped papular lesions on the dorsa of both thumbs (Fig. 4) of 5–6 weeks duration. On direct questioning the patient admitted that he had had a tendency to develop such lesions following any scratch since his early childhood. Later, the development of similar lesions which followed accidental superficial cuts and abrasions at work was closely observed. There was little doubt that they were typical examples of RPC.

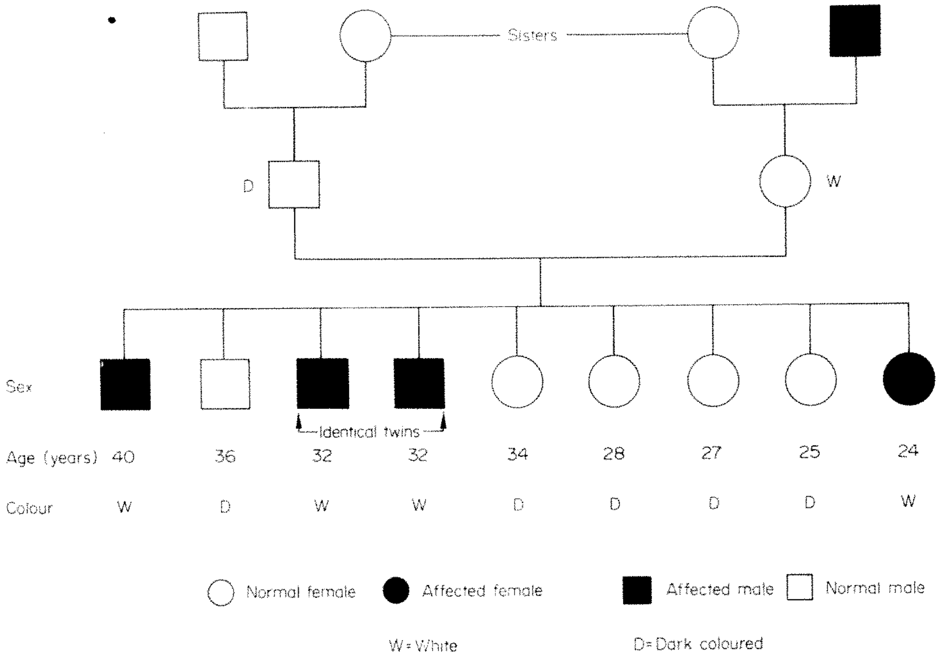

FIGURE 5. Family B: family tree showing affected members and the relevant data.

He also volunteered to have the following two experiments performed. Using a sterile no. 1 gauge hypodermic needle, two parallel linear scratches 15 mm long and 5 mm apart were made on the flexor aspect of the left forearm just deep enough to produce visible bleeding. A close daily follow-up failed to show any lesions suggestive of RPC and healing was almost complete by the tenth day. The other experiment was performed over the knuckle of the right middle finger. Using a 4 mm punch a circular hole 1 mm deep was produced (Fig. 6a). During the following weeks he developed an RPC lesion (Fig. 6b and c) and close follow-up showed complete regression within 10 weeks from the injury. A biopsy obtained at 4 weeks showed histopathological changes comparable to those in accidental RPC.

The patient also stated that his lesions occurred more frequently and took much longer to heal during the winter months. Besides, he used to suffer from chilblains of the hands and feet during the cold season. Like the other patients, he learned to facilitate the quick regression of an RPC lesion by picking off the central plug when it first formed. Routine laboratory examinations of his blood, urine and stools were normal.

The clinical findings in cases 5, 6 and 7 in this family are summarized in Table 1.

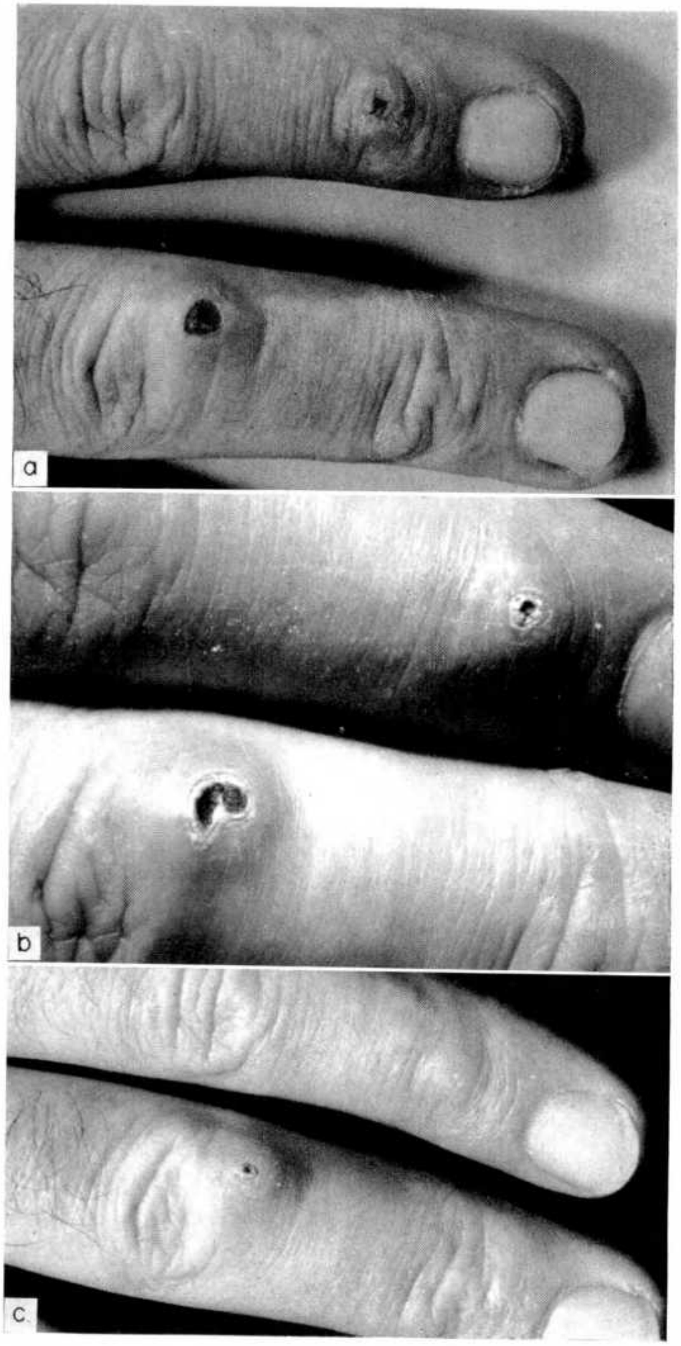

FIGURE 6. (a) An experimental 4 mm punch biopsy 1 mm deep on the right middle finger (case 4) and an early accidental RPC lesion on the right index finger. (b) A mature RPC lesion at the experimental site at 4 weeks. (c) Same lesion at 8 weeks (involution stage).

DISCUSSION

To those who are unaware of this rare skin disorder, RPC may prove difficult to differentiate from similar skin lesions. In fact two siblings described by J. Weiner in 1967 as cases of Kyrle's disease were later shown by Mehregan to be RPC and were included in his review of ten cases (Mehregan, 1970). Other lesions which may mimic RPC were reviewed by A. Weiner (1970) and include molluscum contagiosum, papular urticaria, elastosis perforans serpiginosa and perforating folliculitis described by Mehregan & Coskey in 1968. Worthwhile additions to Weiner's list are Ferguson-Smith's familial self-healing squamous cell carcinomata and the so-called picker's nodule.

The mainstay in the diagnosis of RPC is the demonstration of Van Gieson-positive strands of necrobiotic collagen extruded through the epidermis among other components of the debris in the central umbo of a mature lesion. However, in very early lesions of RPC a haematoxylinophilic mass of necrobiotic collagen can be seen dilating the dermal papilla in the centre of the lesion (Mehregan *et al.*, 1967).

The aetiology of RPC is still a mystery although the defect seems to be genetic. In favour of this concept are the following: the disorder dates back to early childhood in the majority of recorded cases; its occurrence in siblings in two families in this series, in three pairs of siblings in Mehregan's series (1970) and in another pair reported by Weiner (1970); and its restriction in this series to siblings with similar physiognomic features, build and colour. Moreover, in view of the close parental consanguinity in the present series, the involvement of both sexes, the absence of the disorder in the parents and its rarity, an autosomal recessive mode of inheritance seems probable. However, the history of superficial trauma preceding most RPC lesions in reported cases and the successful production of experimental lesions in Bovenmyer's patient (1970) and in two patients in this series leave little doubt about the decisive role of trauma. Nevertheless, Weiner (1970) in his communication on RPC stated that the presumed role of trauma in his two patients had not been observed consistently, and two of the dry-skinned patients in family A' described here, developed RPC lesions on their faces as a result of long exposure to cold winds during the winter months. These facial lesions could be prevented by the daily use of an emollient cream. This observation certainly does not favour such lesions being induced by acne. It does not exclude neurotic excoriations as a provocative factor, although one would expect these to occur during the warm season as well. On the other hand, Bovenmyer (1970) hinted at the occurrence of spontaneous RPC papules at the sites of acne comedones which his patient developed at 10 years of age. He also learned from her mother that the lesions were much improved during the summer months, which she attributed to exposure to the sun and to more rapid loosening of the plugs from soaking in the swimming pool. These observations, together with the undue intolerance to cold in the majority of the patients reported here, the increased frequency and the delay in the regression of their lesions during the cold season suggest that cold could have a significant potentiating role in the provocation of RPC at least in some patients.

As to the pathogenesis of this disorder, RPC lesions are nearly always provoked by a superficial breach of the skin. As Mehregan (Mehregan *et al.*, 1967; Mehregan, 1970) suggested, the first alteration occurs in the form of necrobiosis in the papillary collagen. How and whether cold can potentiate the traumatic factor needs further investigation. Another interesting observation has been the absence of such lesions where the patients sustained deeper skin injuries, such as surgical incisions and accidental deep wounds. This phenomenon suggests that the primary defect lies in the pars papillaris of the dermis. The subsequent reactive epithelial hyperplasia and the formation of perpendicular intraepithelial 'pseudocanals' through which the 'alienated' collagen is eliminated explain the evolution of an RPC lesion and the delay in the healing of the initial abrasion. Such changes add further support to the concept that the epidermis and the pars papillaris are one biological unit (Winter, 1964; Sweet, 1971). According to Mehregan (Mehregan *et al.*, 1967; Mehregan, 1970) RPC represents one of several examples of the phenomenon of transepithelial elimination of foreign particulate and effete material from the dermis. Malak & Kurban (1971), enlarging on this concept, viewed the epidermis as an organ participating actively in the percutaneous elimination of materials for which the term 'epidermal catharsis' was proposed. They also expressed the opinion that the particular *modus operandi* may be dependent on the size and nature of the material to be disposed of. The phenomenon of epidermal catharsis is regarded by them as another manifestation of the intimate dermoepidermal interplay.

Finally, a close follow-up of the patients described here and a review of other reported patients failed to reveal RPC lesions on the palms, soles and hardly any on the trunks. Besides, the unsuccessful attempts at reproducing experimental lesions on the trunk and flexor aspect of the forearm in two of these patients and on the thigh of Bovenmyer's patient suggest a certain topographical predilection for this genetic predisposition. This phenomenon supports the idea that the areas of predilection for RPC are those most exposed to a cold atmosphere.

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Incorporation of D-amphetamine into pigmented guinea-pig hair

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SUMMARY

Incorporation of D-amphetamine into the hair of a black-pigmented guinea-pig was measured by a ^{14}C-tracer method. D-Amphetamine administered intraperitoneally was found to be incorporated into the hair, and part of the radioactivity was found in isolated melanin. The findings are in conformity with the concept that D-amphetamine gains access to melanocytes through the arteriovenous or lymphatic circulation, enters melanosomes, interacts with the melanosomal protein and melanin, and resides ultimately in the pigment granules in the hair fibre. The findings suggest a pathway which may influence the biological activity of this and related drugs.

The structural and chemical similarity of the sympathomimetic amine, D-amphetamine, to L-tyrosine and L-dopa, the precursors of melanin, raises the question as to whether it can gain access to melanocytes and be incorporated into melanin of skin and hair. In a recent study (Yu & Van Scott, 1973), numerous compounds related to L-tyrosine and L-dopa were tested for ability to form pigment in melanocytes of freshly plucked hair of humans and guinea-pigs, and about one-third were found to convert to pigment to varying extents; however, D-amphetamine was not tested.

In a recent study in our laboratories (Harrison, Gray & Solomon, 1974), L-dopa, L-α-methyldopa and DL-isoproterenol were shown by *in vivo* experiments involving radioactive tracers to be incorporated into the hair of black-pigmented guinea-pigs. These drugs were found to be incorporated into both the melanin and non-melanin components of hair, and the results were interpreted as indicating drug association with both the protein and the melanin in the melanosomes. L-dopa was found to be incorporated into the hair of an albino guinea-pig, indicating an independence of the incorporation from active melanogenesis. The results were in conformity with the concept that these drugs gain access to melanocytes through the arteriovenous or lymphatic circulation, enter melanosomes, interact with the melanosomal protein and melanin, and reside ultimately in the pigment granules in the hair fibre. It was considered that melanogenesis in hair may serve well as a model for melanogenesis in other tissues. In other related investigations (Harrison *et al.*, 1973), we have found that melanin of hair and skin has an absorptive capacity for catecholamines and related drugs, therefore necessitating consideration of absorption of drugs by melanin as well as biosynthetic incorporation in interpretation of experiments of this type.

Reported herein are the results of an *in vivo* radioactive tracer study of incorporation of D-amphetamine into melanin and non-melanin components of the hair of a black-pigmented guinea-pig.

MATERIALS AND METHODS

An English smooth hair guinea-pig with black-pigmented hair, skin and eyes was used. The D-amphetamine-2-^{14}C was obtained from Mallinckrodt, Inc. An aqueous solution of the drug was administered intraperitoneally. The solution (1·0 ml) contained 0·8 μmol D-amphetamine and 100 μCi radioactivity; the dose was administered in equal portions (0·25 ml) on each of the first 4 days. The weight of the animal was 255 g. Extrafollicular hair (100–200 mg) was sampled daily for a 35-day period by cutting from numerous areas of the body in a random manner; a final hair sample was taken after 42 days. The daily hair samples were washed in 0.01 M HCl in a Buchner funnel and dried with acetone and air. For total radioactivity in intact hair, 50 mg of the washed hair was combusted in a Packard Tricarb Sample Oxidizer 305. The ^{14}C-labelled CO_2 was trapped and counted in Packard 'Permablend' with a Packard Tricarb Scintillation Counter. In addition, 50 mg samples were placed in sealed vials containing 1·0 ml of 1·0 M HCl and hydrolyzed for 5 days at 100°C in a constant temperature heating block. The acid supernatant was changed daily for the first 3 days, each change involving a centrifugation for 30 min at 1000 *g*; separate experiments showed that this results in the removal of all acid soluble radioactivity. Insoluble matter remaining after 5 days of hydrolysis was

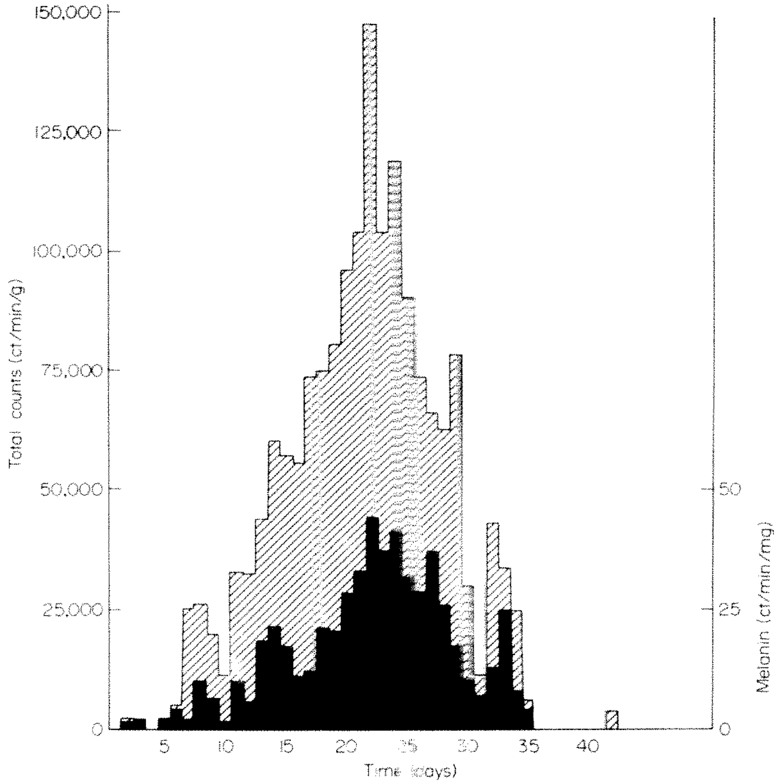

FIGURE 1. Time course of the incorporation of D-amphetamine-^{14}C into pigmented guinea-pig whole hair (hatched area) and isolated melanin (solid area).

operationally defined as melanin; it was collected by centrifugation for 30 min at 1000 *g*, washed and recentrifuged four times with de-ionized water, then transferred to tared filter paper and air dried. After drying, the residues were weighed and analyzed for ^{14}C as described for intact hair.

RESULTS

Incorporation of ^{14}C into the extrafollicular hair of the pigmented animals after intraperitoneal injections of D-amphetamine-^{14}C is shown in Fig. 1 in terms of specific radioactivity of the hair and also of isolated 'melanin'.

DISCUSSION

The ^{14}C incorporation into the extrafollicular hair of the pigmented guinea-pig (Fig. 1) clearly shows that D-amphetamine is incorporated into the structure of the hair and resides in both melanin and non-melanin fractions. The results are similar to those found with L-dopa, α-methyldopa, and isoproterenol in our previous study (Harrison *et al.*, 1974) and are best explained by and conform with the concept that D-amphetamine enters the melanocyte, associates with either or both the protein and melanin components of the melanosomes, and then is carried into the hair shaft by hair bulb cellular activity. The possibility of involvement of keratinocytes in uptake of drugs of this type was not excluded by the experiment and will be studied separately.

We have not ascertained the chemical state of the D-amphetamine in the hair. We do not assume that the melanin-associated radioactivity represents polymerized amphetamine. Numerous reactions, such as hydroxylation by dopamine-β-hydroxylase or tyrosyl hydroxylase and/or absorption or biosynthetic incorporation into melanin could occur; these possibilities are being investigated. The concept of association of the drug with the protein component of melanosomes is speculative and introduced by analogy with the observation by Takahashi & Fitzpatrick (1966) of L-dopa associated with the protein of the melanosomal fraction of mouse melanoma, as demonstrated by the release of L-dopa during acid hydrolysis of isolated melanosomal protein. A definitive conclusion concerning the drug's involvement with protein in our studies and in those of Takahashi & Fitzpatrick must await further clarification.

Another question arises as to whether the *in vivo* uptake of amphetamine into hair is demonstrable in *in vitro* experiments with intact hair or isolated hair melanin. Amphetamine has not been tested in this way, but similar drugs, such as noradrenaline, adrenaline, isoproterenol, octopamine, α-methyldopa, dopamine and L-dopa, have been shown in *in vitro* experiments (Harrison *et al*, 1973) to bind to isolated hair melanin. Also the question arises as to whether amphetamine is incorporated *in vivo* into non-pigmented hair; this has not been tested but we have found in *in vivo* experiments that L-dopa is incorporated to the same extent into albino guinea-pig hair excluding, of course, incorporation into the insoluble melanin fraction which is not present.

The present study with D-amphetamine and our previous work with α-methyldopa and isoproterenol raise similar questions. Assuming that the drug affinity shown for hair melanin also occurs with cutaneous melanin as well as other tissues, as our preliminary data indicate (Harrison *et al.*, 1973), prolonged use of the drug might alter the function of the melanin, particularly during pre- or post-natal development. We are performing long-term experiments to determine this.

Our studies suggest that hair may be a useful and sensitive indicator of past consumption by humans of amphetamine and related drugs, since the hair cycle of the human may span up to several decades. This would be analagous to the analyses of arsenate and various metals in hair which have proved extremely valuable for establishing different types of poisoning. The D-amphetamine as well as the drugs previously studied (Harrison *et al.*, 1973) are incorporated into the hair from either the arterio-venous or lymphatic circulation and therefore the drug entrapment by melanin would reflect the blood level of the drug, and drug location in the hair shaft may indicate the chronology of drug consumption. To exploit this phenomenon further, the drug derivative deposited in the hair must first be characterized and a sensitive method for its analysis in hair developed.

This experiment indicates a pathway for D-amphetamine not considered heretofore in metabolic studies. However minor as a pathway it should not be ignored because it suggests the possibility that long-lasting deposits of drug may exist associated with melanin with unknown consequences to the host.

ACKNOWLEDGMENTS

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Tongue lesions in generalized pustular psoriasis

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SUMMARY

The appearance of the tongue has been recorded in ten consecutive patients suffering from generalized pustular psoriasis and in 1000 consecutive dermatology patients. Tongue lesions similar to geographic tongue (benign migratory glossitis) are frequently seen in patients suffering from generalized pustular psoriasis and an unusually high proportion of patients with generalized pustular psoriasis also have heavily fissured tongues. Moreover, it seems probable that the presence of geographic tongue or heavy fissuring of the tongue in an otherwise apparently normal individual indicates that he or she may possess a propensity to develop generalized pustular psoriasis.

Following the observation of exuberant lesions of geographic tongue (benign migratory glossitis) in a patient suffering from generalized pustular psoriasis (GPP) in 1967, the tongue was inspected, and its appearance recorded, in all patients suffering from GPP seen from then onwards. Nine patients suffering from classical GPP have now been examined, and to this number it has seemed legitimate to add a tenth whose disease might have been termed impetigo herpetiformis as it occurred during pregnancy, but which apart from this circumstance was entirely characteristic of GPP. Geographic tongue was not diagnosed unless there was some elevation of the advancing edge of the lesions, in keeping with the hypothesis that the epidermal spongiform pustule is a characteristic feature of this condition (Gastou, 1902; Dawson, 1969). The salient clinical features of the ten patients mentione above are summarized in Table 1.

It is seen that eight out of the ten patients showed lesions of geographic tongue at some time during the period they have been availabe for examination. In general the more severe the skin lesions, the more florid were the lesions of geographic tongue. Four of the patients with geographic tongue (cases 5, 6, 8 and 10) were able to say that the condition had been present for many years before the onset of GPP. Two of the patients (cases 1 and 10) were examined when no trace of psoriasis of any type was present on the skin, yet well-marked lesions of geographic tongue were noted at that time. In another two patients (cases 5 and 6), geographic tongue was observed when no pustular lesions were present on the skin, though lesions of non-pustular psoriasis were present to a slight extent. In the remaining cases various degrees of pustulation were present on the skin when geographic tongue was observed; in the case of patient 4 this at times amounted to no more than palmoplantar pustulosis.

Three of the patients with GPP and geographic tongue also had fissured tongue. (For the purpose of this article fissured tongue refers only to heavy fissuring affecting the greater part of the dorsal surface.)

TABLE I. Clinical features of patients with generalized pustular psoriasis (GPP)

Patient no.	Sex	Date of birth	Psoriasis, apart from GPP, with date of onset	Generalized pustular psoriasis with date of onset	Fissured tongue	Geographic tongue
1	F	1897	1967. Discoid	1969, 1972	No	Geographic tongue observed during and between attacks of GPP. Patient unable to say when geographic tongue first appeared
2	F	1901	Unable to give history	1967, 1968, 1969, 1970	Very slight	Geographic tongue observed during attacks of GPP. Patient unable to give any history
3	M	1903	1932. Discoid	1972	No	No history of geographic tongue. Tongue appeared somewhat redder than normal during GPP but no geographia observed
4	M	1904	1922. Discoid and palmoplantar pustulosis	1956, 1967, 1973	Heavily fissured	Geographic tongue observed during and between attacks of GPP. Between attacks lesions of geographic tongue minimal. Patient unaware he had geographic tongue
5	F	1910	1965. Discoid	1971, 1972	Very slight	Well marked lesions of geographic tongue observed during and between attacks of GPP. Patient aware of the presence of geographic tongue since age of 20 years
6	F	1921	1953. Flexural	1953. Six attacks since, separated by intervals of several years	Heavily fissured	Geographic tongue observed during and between attacks of GPP. Lesions of geographic tongue much less marked when psoriasis in remission. Patient confident geographic tongue present as long as she can remember
7	F	1924	1960. Discoid	1971	No	No history of geographic tongue. Geographic tongue not observed during or followingGPP
8	F	1937	1950. Discoid	1960, 1963, 1967, 1970	No	Geographic tongue observed during GPP. Patient certain geographic tongue present intermittently since early childhood

Table 1 (*cont.*)

9	F	1948	1956. Acrodermatitis continua of Hallopeau	1972	Heavily fissured	Geographic tongue observed during GPP. Patient cannot say when geographic tongue first appeared
10	F	1950	Never	1972 (during pregnancy)	Very slight	Well marked geographic tongue observed during GPP and on review 6 months and 1 year later when no skin disease present. Patient aware of the presence of geographic tongue since the age of 16 years

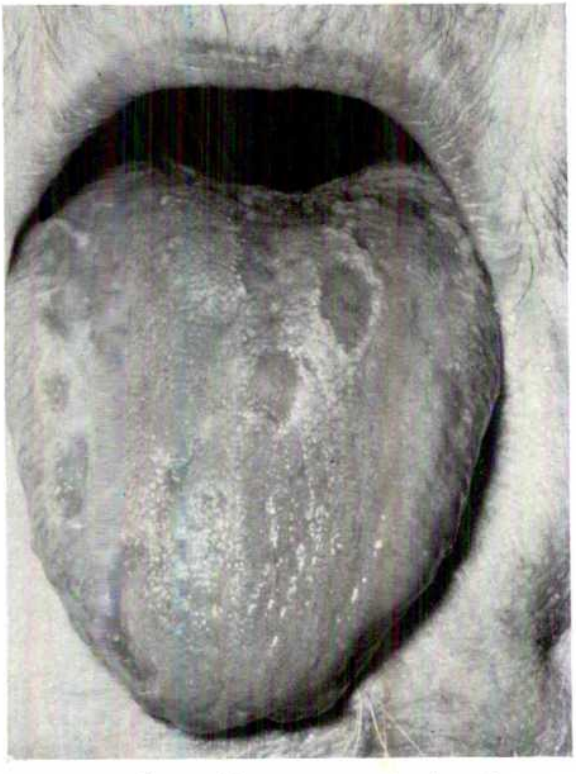

FIGURE 1. Geographic tongue occurring in patient 1.

Figs. 1 and 2 illustrate tongues with representative lesions.

In order to compare the prevalence of geographic tongue and fissured tongue in GPP with their prevalences in skin disease in general, and with psoriasis in particular, the tongue was personally examined in 1000 strictly consecutive patients attending dermatology out-patient clinics or after admission to the dermatology in-patient unit. Table 2 sets out the result of this investigation in respect of the entire series, and Table 3 shows the prevalences in respect of psoriasis. No distinction was made between types of psoriasis, so that a patient seen with any type of psoriasis, including GPP, was represented in the series. In fact four patients (cases 1, 4, 5 and 6) who had suffered from

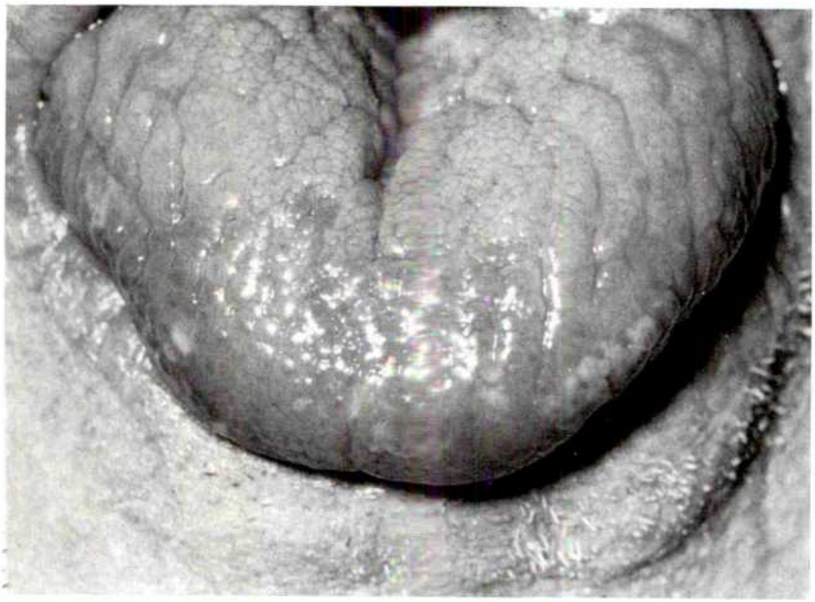

FIGURE 2. Heavily fissured tongue of patient 4 with minimal lesions of geographic tongue towards the tip.

TABLE 2. Prevalence of geographic tongue and fissured tongue in 1000 consecutive dermatology patients

Sex	Total no. of patients (mean age in years ± SD)	Geographic tongue alone	Geographia with or without fissuring	Geographia and fisuring	Fissuring with or without geographia	Fissured tongue alone
Males	417 (36 ± 23)	2	11	9	23	14
Females	583 (34 ± 22)	9	13	4	23	19
Total	1000 (35 ± 22)	11	24	13	46	33

GPP and had tongue lesions were included as they were reviewed while the survey was being carried out.

DISCUSSION

Consideration of the data presented in Tables 1, 2 and 3 strongly suggests that there is an increase in the prevalence of geographic tongue and fissured tongue in patients with GPP as compared with patients with skin disease in general, or with psoriasis in particular.

It might be argued that the tongue lesions referred to in patients with GPP were not in fact geographic tongue. Clinically, however, they are similar, though the lesions are perhaps rather more

TABLE 3. Prevalence of geographic tongue and fissured tongue in 117 consecutive psoriatic patients

Sex	Total no. of patients (mean age in years ± SD)	Geographic tongue alone	Geographia with or without fissuring	Geographia and fissuring	Fissuring with or without geographia	Fissured tongue alone
Males	50 (36 ± 23)	0	3	3	5	2
Females	67 (29 ± 21)	4	5	1	2	1
Total	117 (32 ± 22)	4 (3·4%)	8 (6·8%)	4 (3·4%)	7 (6·0%)	3 (2·6%)

active in GPP than in other cases, while histologically the tongue lesions of GPP and geographic tongue have been reported to be identical (O'Keefe, Braverman & Cohen, 1973). Again, the history, confidently given by four out of the ten patients with GPP, that geographic tongue had been present for many years before the onset of psoriasis of any kind, and the personal observation of geographic tongue in four out of the ten patients with GPP when no pustular lesions were present on the skin, suggest that the lingual lesions seen in these cases could not be entirely explained as manifestations of GPP.

The literature contains numerous reports of mucosal lesions occurring in GPP and occasionally in other types of psoriasis, yet few authors mention the possibility of an association between geographic tongue, fissured tongue and GPP. Among those who have, Schuppener & Kober (1957) referred to the presence of heavy fissuring of the tongue in two patients suffering from GPP. One of these patients also had geographic tongue, and both developed an exfoliative glossitis at the height of the illness. Schuppener (1960) included geographic tongue as a manifestation of GPP. Chorazak, Pietrzykowska & Smigla (1964) reported three patients with atypical (but not GPP) psoriasis who also had heavily fissured and geographic tongues and concluded that the association was significant. O'Keefe *et al.* (1973) reported three patients with pustular psoriasis and another with pityriasis rubra pilaris, all of whom had lingual lesions similar to geographic tongue.

The occurrence of lesions similar to geographic tongue in Reiter's disease has been referred to by Hancock (1960) and Pindborg, Gorlin & Asboe-Hansen (1963).

Pindborg (1968) stated that geographic tongue occurs more frequently in patients with psoriasis than in other subjects but did not quote any statistical evidence. Some support for the validity of Pindborg's statement may, however, be gathered from a comparison of the prevalence of geographic tongue among patients suffering from psoriasis of any type (including GPP) and dermatology patients in general, as shown in Tables 2 and 3.

Besnier (1902) apparently believed there was an association between geographic tongue and seborrhoeic eczema. Interestingly enough, Rahamimoff & Muhsam (1957), in an investigation involving more than 1000 children with geographic tongue in Israel, did report a positive correlation ($r = 0{\cdot}60$) between geographic tongue and seborrhoeic eczema.

A strong association between geographic tongue and fissured tongue has been well documented (Gubler, 1869; Caratzali, 1933; Berggreen, 1936; Turpin & Caratzali, 1936; Turpin, 1947; Shuermann, Greither & Hornstein, 1966) and is again clearly demonstrated by inspection of Tables 2 and 3.

Heredity has been cited as a major aetiological factor in some cases at least of geographic tongue (Gubler, 1869; Bridou, 1872; Caratzali, 1933; Turpin & Caratzali, 1936; Turpin, 1947; Rahamimoff

& Muhsam, 1957; Dawson & Pielou, 1967) and similarly of fissured tongue (Caratzali, 1933; Turpin & Caratzali, 1933, 1936; Tobias, 1945; Turpin, 1947; Rahamimoff & Muhsam, 1957).

Lapière (1961) argued that pustular psoriasis, impetigo herpetiformis, acrodermatitis continua and Reiter's disease are all closely related, genetically dependent and that the common pathological factor is the epidermal spongiform pustule. He also suggested that the epidermal spongiform pustule is a giant Munro abscess which develops only in subjects who are genetically psoriatic. Dawson (1969) added geographic tongue to the list of conditions in which an epidermal spongiform pustule occurs. It therefore seems possible that there is a certain type of genetic inheritance which can manifest itself as geographic and fissured tongue and also bestow a propensity on the individual concerned to suffer from GPP.

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Skin reactions to primary irritants and predisposition to eczema

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SUMMARY

Fifty individuals with healed hand eczema were tested with nine primary irritants. The results were compared with those of carefully matched controls. An increased skin reactivity to primary irritants was not demonstrated in the 'eczematous subjects'. The value of testing with irritants in pre-employment evaluation could not be confirmed.

Is it possible to predict an individual's predisposition to eczema by testing the normal skin with primary irritants? Do patients with healed hand eczema have any altered skin reactivity once their eczema is healed and therefore can no longer influence the general skin reactivity? These questions are highly relevant to pre-employment testing.

MATERIAL AND METHODS

The patients and controls were chosen mainly from occupational groups such as housewives, sanatory personnel, hairdressers, metal workers and dye workers.

Patients

Fifty patients with healed hand eczema were selected for the study. The diagnosis had been made by experienced doctors in the Department of Dermatology, Gothenburg. The eczema had been quiescent for 1 month in one patient, 2 months in two patients and 3 months or more in forty-seven patients. It had been clinically healed for 1 year or more in twenty-eight and 2 years or more in fifteen patients. All types of hand eczema diagnosed morphologically were included except vesicular pompholyx and atopic dermatitis. It would of course have been desirable to evaluate the skin reactivity in separate groups of allergic and toxic contact dermatitis. However, as the diagnosis of toxic eczema must be made by exclusion of an allergy this diagnosis would often have been uncertain (Fregert & Hjorth, 1968).

Controls

The controls were selected so that they were matched to the patients to be of the same sex, of the same age (differences in age did not exceed 5 years) and of the same occupation (in regard to the contact with primary irritants and/or allergens). Other factors, which may influence the basic skin reactivity, were also evaluated and there were no statistically significant differences between the patients with healed hand eczema and their controls regarding the colour of their skin, tanning from the sun, greasiness of the skin (judged clinically), earlier and co-existing skin or other diseases, or of drugs being taken at the time of testing.

Test sites

All the patients and controls were tested in exactly the same areas on the fronts of their thighs. The skin on the test site was macroscopically normal, it had not previously been affected by skin lesions and had not been washed or shaved during the 24 h prior to the tests.

Test methods

Cup tests (Blohm, 1960) were performed with potassium hydroxide (0·05, 0·09, 0·14 and 0·17% in distilled water), exposure time 2 h and reading after 24 and 72 h.

Patch tests (adhesion chamber method) (Rokstad, 1940) were performed with sodium lauryl sulphate (20, 3, 1 and 0·5% in distilled water), benzalkonium chloride (2, 1·5, 1 and 0·1% in distilled water), potash soap (50, 30, 10 and 5% in vaseline), cantharidin (0·01, 0·007, 0·005, 0·004, 0·003 and 0·002% in acetone) and croton oil (0·5, 0·4, 0·3 and 0·2% in vaseline). For cantharidin a cellulose patch test was used.

All patches in these serial dilutions were removed after 24 h, the first reading was done 15 min later, and a second reading after another 48 h.

Open tests (Wedroff & Dolgoff, 1935) with phenol (20, 15, 12, 10, 7·5 and 3% in distilled water), and trichloracetic acid (30, 20, 10 and 7·5% in distilled water) were read immediately after application as well as after 24 and 72 h.

All individuals tested were exposed to the same amount of each test substance.

A numerical grading was used to express the intensity of the skin reactions as judged by the clinical appearance of the test site. Separate index scales (0–7) had to be used to describe the morphology of the reactions for the different irritants, low numbers meaning weak and high numbers more severe reactions (Björnberg, 1968).

A separate study was performed to evaluate the correlation between the macroscopic intensity of the skin reactions and the severity of the histological changes in the test site. These corresponded fairly well (Björnberg, 1968).

Statistics

The *t*-test was used to evaluate the differences between the patients' index values and the values of their matched controls. The error of the methods was estimated according to Dahlberg (1940). The level of significance was 0.05.

Error of the methods

The error of measurements was estimated in a separate series of about twenty-five individuals in each test method for all substances and concentrations. The error was approximately 10–15%. The type of occlusive adhesive plaster and the pressure on the test areas were shown not to influence the test results in the present investigation.

RESULTS

No statistically significant differences between the intensities of the skin reactions to the irritants could be demonstrated between the patients with healed hand eczema and their matched controls in the total of thirty-six comparisons. The exceptions were two weak concentrations of phenol, to which the eczema patients reacted more strongly than the controls.

Thus no generalized increased skin reactivity to the tests with primary irritants could be demonstrated in the 'eczematous' as compared to the 'non-eczematous' subjects.

DISCUSSION

Klauder & Brill (1947) estimated the tolerance of the skin by patch testing with a number of solvents. They considered the tests practical for the selection of persons for work in which there was exposure to petroleum solvents. Carrié (1957) used a standard series of nineteen irritant substances as a pre-employment investigation of the function of the skin. The concentration of the substances was chosen so that they gave toxic reactions. One or more reactions to these substances indicated an increased skin sensitivity in the individual, who might therefore be advised not to start an occupation which was potentially hazardous to the skin. Weitgasser (1958) also used primary irritants to test subjects who intended to start to work in which there were 'skin stressing' conditions.

In the present investigation the value of pre-employment tests with irritants could not be confirmed as the tests with different concentrations of the nine primary irritants did not reveal any increased reactivity in patients with hand eczema, once the eczema was healed.

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A survey of the treatment of 200 cases of basal cell carcinoma (1959-1966 inclusive)

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SUMMARY

The most satisfactory regime for radiotherapeutic treatment of basal cell carcinoma in this study was ten to twelve daily fractions of 400–500 rad, 5 days a week to a total dose of 4000–5000 rad. This method produced the highest cure rate with the least incidence of radionecrosis and the best cosmetic result.

The use of implant methods is retained for lesions in difficult sites, i.e. the inner canthus and the nasolabial folds.

Two hundred cases of histologically proven basal cell carcinoma treated between 1959 and 1966 have been reviewed. These cases were consecutive with the exception of those lacking histological confirmation or adequate follow-up. The age and sex of the patients, the site and size of the lesions, details of treatment and follow-up were recorded. Length of follow-up ranged from 1 to 10 years (Table 1), difficulties being encountered because of the advanced age of many of the patients and the unreliability of postal follow-up, which was undertaken in old patients and in those living far from the hospital. Thirty-one patients died of other causes during the period of study and follow-up, seventeen from other primary carcinomas and one from lymphosarcoma. Twenty-one patients developed a further basal cell carcinoma in a new site.

TABLE 1. Follow-up periods

Follow-up in years	1	2	3	4	5	6	7	8	9	10
No. of cases	29	16	24	29	44	18	17	9	6	8

The aim of the study was to assess the modes of treatment employed with a view to determining the regime that gave the highest cure rate with the least incidence of complications. Treatment was by excision in thirty-five patients, implantation of radium needles or radon seeds in thirty-six or by external radiotherapy in 129. The radiotherapy treatments were characterized by a wide variety of dosage regimes but constant factors were 80 kV and a focal skin distance (FSD) of 30 cm.

Age incidence

Of the 200 cases, 116 were males and eighty-four females. The average age of the patients treated was 60 years, ranging from 20 to 84 years. The maximum age incidence was equally distributed between the sixth and seventh decades.

Size of lesions

The size of the lesions ranged from 0·5 to 10 cm, 82% of patients having lesions of 2·0 cm or less in diameter and 89% of patients having lesions of 3·0 cm or less. The average size for the whole group was 1·54 cm. The average size of the lesions that recurred was 1·3 cm, as was the average size of the treated lesions which were subsequently the site of radionecrosis. All lesions were superficial and not penetrating subcutaneous tissue.

Methods of treatment

(1) 129 patients were treated by superficial irradiation with low voltage X-rays at 80 kV and 30 cm FSD (Table 2).

TABLE 2. Recurrences after irradiation therapy

No. of patients	Daily dosage (rad)	No. of fractions	Total dose (rad)	Dosage in ret	Recurrences	Radio-necrosis
4	250	30	7500	2200	1	—
3	330	18–20	5400–6000	1880–2050	—	—
8	400	10–12	4000–4800	1710–2020	—	—
19	500	12	6000	2440	1	1
87	600	10	6000	2650	6	—
4	750	8	6000	2860	—	2
1	800	5	4000	2330	—	—
3*	1000	3–4	3000–4000	1650–2150	—	1

* This group was treated by 1000 rad at weekly intervals on three to four occasions. All the other patients were treated by a daily fractionation regime.

(2) Of thirty-six patients treated by implant of radium needles or radon seeds to a dose of 6000–8000 rad over an average period of 1 week, three patients had recurrence and four developed radionecrosis.
(3) Of thirty-five patients treated by primary excision of the tumour, one only had recurrence.
(4) Altogether twelve patients either failed to respond to the initial treatment measure or their disease recurred (Table 3).

DISCUSSION

One hundred and twenty-nine patients were treated by superficial 80 kV X-rays, the largest group of eighty-seven receiving 600 rad in ten daily fractions over 2 weeks. This high dose level might be expected to give the greatest cure rate and it is therefore surprising that in six patients the disease recurred or failed to respond: one failed to respond initially; one recurred within 1 year; 1 recurred at 2 years; two recurred after 4 years; and one after 5 years.

A radiation dose of this magnitude would be expected to have a considerable tissue damaging effect but none developed radionecrosis. This particular regime can be considered disappointing in producing a cure rate of only 93%, or a 92% 5-year cure rate if calculated by the actuarial method of Berkson & Gage (1950) to allow for cases with a short follow-up. As a result of this, the regime has been discontinued in favour of a trial of ten to twelve daily fractions of 400–500 rad depending on lesion size, the larger fractions being used for smaller treatment areas. In the twenty-six patients

TABLE 3. Details of recurrences

Case	Site	Size (cm)	Treatment	Time of recurrence
1	Left cheek	2·0	250 rad × 30	Never regressed
2	Right chin	1·0	500 rad × 10	3 years
3	Right zygoma	1·5	600 rad × 10	Never regressed
4	Left neck	1·5	600 rad × 10	Within 1 year
5	Bridge of nose	Unrecorded	600 rad × 11	2 years
6	Right nose	1·0	600 rad × 10	4 years
7	Left inner canthus	1·0	600 rad × 10	4 years
8	Right temple	1·0	600 rad × 10	5 years
9	Left naris	1·0	Radon seed implant	3 months
10	Right forehead	1·0	Radon seed implant	Within 2 years
11	Anterior left ear	Unrecorded	Excision	1 month
12	Left cheek	2·0	Radon implant	1 year

treated at this rate, there was only one recurrence occurring after 3 years and one radionecrosis, giving a cure rate of 96% (or 94%, after 5 years by the actuarial method). No statistically significant difference could be found between patients treated with a daily dose of 500 rad and those treated with 600 rad daily, or between all those treated with 600 rad or more daily and all those treated with less than 600 rad. This may well be due to the small numbers involved. However, combination of the highest cure rate with excellent cosmetic results encourages us to select a dosage of ten to twelve daily fractions of 400–500 rad as the basis for future treatment of these lesions.

Various other treatment regimes have been tried in a small group of eighteen patients. Amongst these it is noticeable that radionecrosis was associated with the large dose fraction treatments: one of three patients receiving three to four fractions of 1000 rad (weekly treatments); two of four patients receiving eight fractions of 750 rad (daily treatments).

Radionecrosis was a problem amongst the patients treated by implant of radium needles or radon seeds to doses of 6000–8000 rad over an average period of 1 week. This particular method of treatment was chosen for lesions in difficult situations, the inner canthus or the nasolabial folds, making them less accessible to treatment by homogenous superficial radiotherapy or excision. In spite of this incidence of necrosis, it is proposed to continue this mode of treatment in these situations. The overall cure rate in this group was 92%.

The number of patients developing radionecrosis was very small, making it impossible to draw any firm conclusions. However, two observations can be made from Table 4. Firstly, the average age of these patients was 54 years as compared with 60 years for the whole study, eliminating senile atrophic skin as a predisposing factor in the development of radionecrosis. Secondly, the average size of the lesions was 1·31 cm as compared with 1·54 cm for all lesions treated, so that large size is not a predisposing factor *per se*. On the other hand, no cases of radionecrosis were observed when the radiotherapeutic dosage was below 2150 ret and it would appear that the dosage in ret is the critical factor in the development of radionecrosis. Large weekly doses of 1000 rad are to be avoided except in special circumstances where age, infirmity or travelling difficulties preclude daily treatment.

The good results obtained in the patients treated by surgical excision may be partly due to case selection, in that small and insignificant lesions were treated most conveniently by this method.

It is of interest to compare our results with those in other series (Table 5), an overall cure rate of 94% comparing favourably with the figures published from other centres using a variety of different treatments.

TABLE 4. Patients developing radionecrosis

Age (years)	Sex	Site	Size (cm)	Method of Radiation	Dosage in ret
52	F	Posterior neck	3·0	500 rad × 12	2440
64	F	Umbilicus	3·5	750 rad × 8	2860
64	M	Scalp	2·0	750 rad × 8	2860
41	M	Right nose	0·5	1000 rad × 3·5	2150
51	M	Right temple		Seeds (6000 rad) (Radon)	
49	M	Left inner canthus		Seeds (6300 rad) (Radon)	
56	M	Right inner canthus		Seeds (6750 rad) (Radon)	
Average age: 54 years			71% males	Average size: 1·31 cm	

TABLE 5. Chart of published results

Author	Years of study	No. of patients	Follow-up period (years)	kV	Cure rate (%)
Radiotherapy					
Ebbehøj (1951)	1936–1951	174	5	12 and 26	96·2
Hultberg (1943)	1943	340	5	30 and 60	90·3
Hansen & Jensen (1968)	1937–1955	1198	10	30 and 60	83·6
Mosekilde	1951	603	5	26	90·4
Johansen (1961)	1961	334	5	30	96
Nevrkla & Newton (present series)	1959–1966	129	1–10	80	94
Radium needles					
Nielsen (1933)	1928–1933	—	—	—	70–75
McAuley (1963)	1963	18	1–10	—	100
Nevrkla & Newton (present series)	1959–1966	36	1–10	—	94
Moh's chemotherapy					
Tromovitch, Beirne & Beirne (1966)	1966	102	5	—	93·1
Phelan (1968)	1968	339	1–5	—	93
Morgenstern & Leeper (1964)	1964	186	1–8	—	100
Mohs (1956)	1956	267	5	—	95·6
Curettage and cauterization					
Simpson (1966)	1966	835	2–5	—	93
Sweet (1963)	1963	460	2–5	—	93
Nelson (1966)	1965	410	1–5	—	94·9
Excision					
Freeman, Knox & Heaton (1964)	1964	1341		—	84
Nevrkla & Newton (present series)	1959–1966	35	1–10	—	97

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An unusual papular and acneiform facial eruption in the negro child

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SUMMARY

An unusual facial eruption occurring in twenty-two negro children is described. The cause is obscure but certain aetiological factors are discussed.

In the course of the last 3 years we have seen twenty-two negro children with a distinctive papular eruption involving the face, which does not seem to have been described previously. The object of this report is to draw attention to a condition which we believe is not rare and is easy to diagnose once a clinical features have been appreciated.

CLINICAL PICTURE

All the twenty-two children suffering from this condition have been negro (twenty-one West Indian and one Nigerian) and most have been born in this country. We have never seen a similar eruption in a European child.

Appearances

The rash was confined to the face and ears and consisted of small rounded papules 1–3 mm in diameter. Usually the papules were flesh-coloured but were hypopigmented in some cases. They tended to be scattered rather than grouped, the cheeks being affected in all patients. A particular feature of this condition appeared to be involvement of the upper eyelids and helices of the ears (Figs. 1 and 2). No pustules were seen and comedones were found in only three cases. In some children mild eczema was present on the face but was not a prominent feature and appeared to be unrelated to the papular lesions.

Age at onset

This has varied between 9 months and 12 years with a mean age of 5 years, and boys (fourteen) were affected more often than girls (eight).

Familial Incidence

In four families, more than one member of a family was affected, but single cases have also been seen in ten families.

Course of the disease

This is unpredictable and usually the rash is slow to clear. Only two cases have resolved within 6 months, while in the remainder the rash has persisted and in some has still been present after 3 years. To date we have not seen this condition in the adult, but perhaps it is too early to assume that all cases clear by puberty.

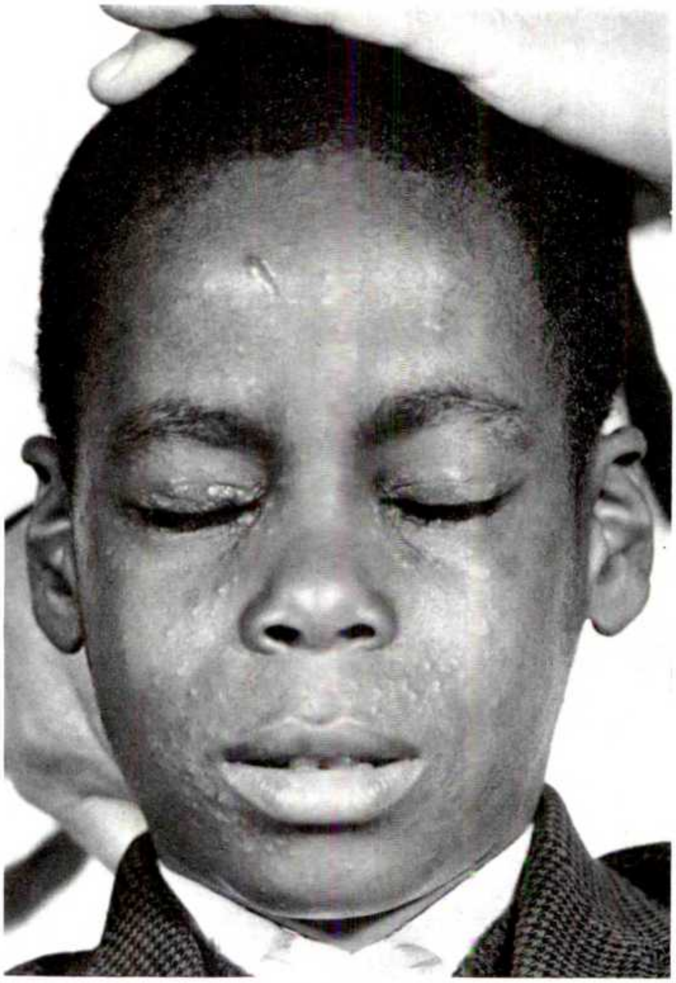

FIGURE 1.

Associated findings

In twelve patients who presented with the facial eruption, mild eczema was noted in three, while a further nine children attending with eczema were found to have the characteristic facial rash. Thus twelve children had both conditions but there was no correlation between the course of the eczema and that of the facial eruption.

Investigations

Investigations including full blood counts and tests for sickle cell disease have all been normal and no evidence of Demodex infection has been found.

Histology

Papules from eight patients were examined histologically. The abnormal features were as follows. All eight showed mild hyperkeratosis, with either minimal or no parakeratosis, and minimal or no acanthosis. In seven cases there was no evidence of spongiosis. All showed in the dermis a mild to moderate perivascular infiltrate, predominantly lymphocytes and histiocytes with a lesser number of plasma cells and only a very occasional granulocyte. In one case there was a central focus of hyperkeratosis and parakeratosis with spongiosis, and in the dermis beneath this area the infiltrate was fairly uniform and had lost its focal character. In no biopsy was any abnormality of the follicle seen. Although the abnormal features were not specific, they did show a similar pattern in all biopsies.

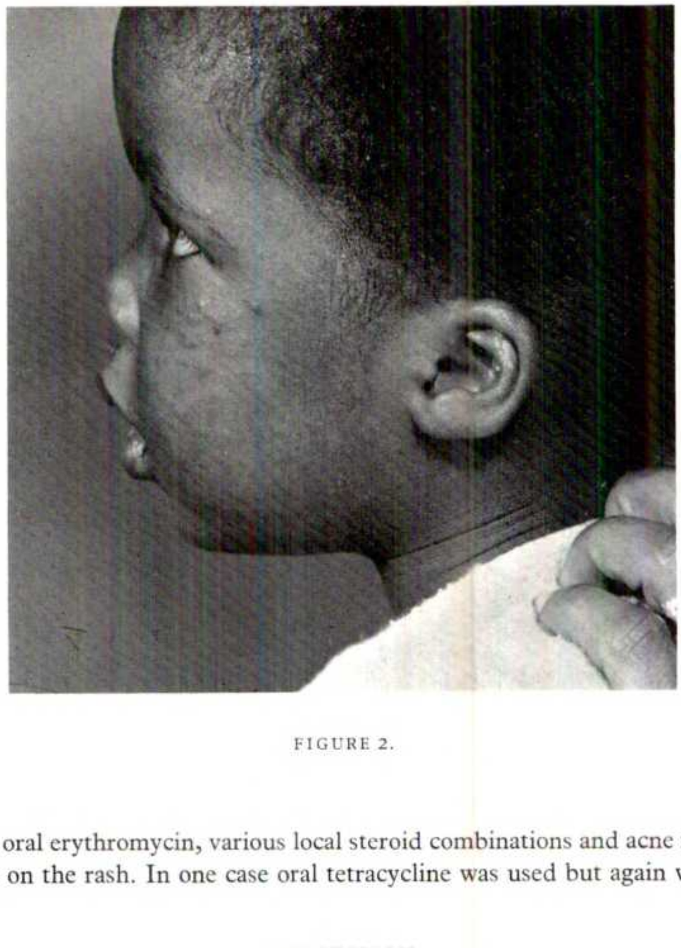

FIGURE 2.

Treatment

This has included oral erythromycin, various local steroid combinations and acne remedies, but none has had any effect on the rash. In one case oral tetracycline was used but again without benefit.

DISCUSSION

The facial eruption which we have observed in these twenty-two negro children appears to be a definite entity. In all cases the rash was strikingly similar and once one was aware of the condition the diagnosis was not difficult. In some families several children showed the rash while in others only one child was affected. The aetiology of the condition is by no means clear. We do not think this is merely a variant of eczema despite the fact that in twelve of the children eczema was present on other parts of the body; the remaining ten showed no evidence of eczema.

The use of various oils and greases on the skin and hair is common practice amongst West Indians and clearly might have had aetiological significance, but only ten of the children had used any vaseline or Johnson's Baby Lotion. In all cases stopping these preparations made no difference to the course of the facial eruption. None of the children had used brilliantine and no oils were used during any hair straightening procedures. In pomade acne (Berlin, 1954; Plewig, Fulton & Kligman, 1970) the eruption is concentrated on the cheeks, temples and sometimes on the ear lobes, but the eyelids are not affected. Involvement of the eyelids has been a common and characteristic feature in our cases.

The histological features are not those of a focal follicular lesion, and this is further evidence against this being a type of oil acne.

The clinical picture was quite unlike ordinary infantile acne and none of the children were taking any drugs which might have caused this eruption. The prior use of local steroid preparations did not appear to have any aetiological significance, as in a number of cases no local treatment had been used. In those children where this treatment had been prescribed there was no obvious effect on the rash, and stopping such treatment produced no improvement in the eruption.

Although the first clinical impression is the similarity to acne, the age range, clinical distribution and histology are against this as a definitive diagnosis. We have not found any cause for this eruption, but perhaps some external agent may still ultimately be shown to be responsible, although none has been found in our series.

ACKNOWLEDGMENTS

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Pharmacology and Treatment

Palmar eccrine sweating—the role of adrenergic and cholinergic mediators

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SUMMARY

Fifty-one healthy human volunteers received intradermal injections of adrenaline. Sixty-five per cent of injected forearms and 36% of injected palms showed a positive sudorific response. Adrenaline-induced palmar sweating was not inhibited by atropine blockade or local anaesthesia. Pharmacologically proven atropine blockade completely suppressed emotional palmar sweating, but had no effect on spontaneous palmar sweating.

Three groups of five healthy human volunteers were given intravenous doses of: (1) the alpha-blocker, phentolamine (Rogitine); (2) the alpha-blocker, phenoxybenzamine (Dibenyline); and (3) the beta-blocker, propranolol (Inderal). The blocking agents did not inhibit palmar sweating caused by either emotional stimuli or intradermal adrenaline, and did not suppress spontaneous (base line) sweating of the palmar glands.

The failure of atropine to block spontaneous (or baseline) palmar sweating may represent either another pharmacological mediator or non-pharmacologically mediated eccrine gland water loss. If the former is the case, then these findings suggest that the palmar eccrine gland may be related to neither alpha- nor beta-receptors, but rather to a previously undescribed type of adrenergic receptor.

The physiological control of the palmar eccrine sweat gland is still uncertain. Although the gland is unquestionably stimulated by cholinergic mediators (Dale & Feldberg, 1934; Gordon & Maibach, 1966; Goodman & Gilman, 1965), the role of adrenergic stimuli is controversial (Haimovici, 1950; Randall & Kimura, 1955; Gordon & Maibach, 1965; Foster & Weiner, 1970).

In support of adrenergic mediation of eccrine sweating, Haimovici (1950), utilizing a tannic acid-ferric chloride colorimetric determination, observed a decrease in palmar sweating following the intravenous (i.v.) administration of the alpha-adrenergic blocking agent Dibenamine (N,N-dibenzyl-β-chloroethylamine). Further supportive evidence was advanced by the observation that intradermally injected catecholamines induced eccrine sweating (Barnett, 1951; Chalmers & Keele, 1951; Dutta, 1967; Hurley & Witkowski, 1961).

In this study we have employed the modified Bullard sudorometer (Gordon & Maibach, 1966) to record kinetically the sudorific effects of adrenergic and cholinergic mediators. First, we have observed the direct actions of adrenaline and acetylcholine, alone and following both atropinization and lignocaine local anaesthesia. Next, we have demonstrated the effects of alpha- and beta-adrenergic and atropine-induced cholinergic blockade upon spontaneous and emotional palmar sweating.

MATERIALS AND METHODS

Fifty-one healthy human volunteers (male and female, age range 19–58 years) received intradermal injections of 0.1 ml of adrenaline 1:1000. The site of injection was the volar surface of the forearm in twenty-three subjects and the palm in a further twenty-eight. In each study the modified Bullard sudorometer was employed to ascertain base line (spontaneous) eccrine sweating and to monitor the effects of intradermal adrenaline. As a control for emotionally-induced sweating, the contralateral palm or forearm received an intradermal injection of 0.1 ml of phosphate-buffered saline (pH 7.4).

Five positively responding palms and five positive forearms were re-injected with intradermal adrenaline following blockade with atropine sulphate (0.4 mg intradermally). Satisfactory cholinergic blockade was demonstrated by injection of 0.1 ml of methacholine (Mecholyl) 1:1000 (Gordon & Maibach, 1966). Three positive palms and three positive forearms also received repeat injections of adrenaline following local anaesthesia with lignocaine.

In fifteen subjects we studied the effects of cholinergic blockade on spontaneous and emotional palmar sweating. Spontaneous palmar sweating and the effects of various emotional stimuli (mental arithmetic, intradermal saline, intramuscular saline, threatening conversation) were recorded before and after blockade with atropine sulphate (0.4–0.8 mg intradermally by injection or iontophoresis). Adequacy of cholinergic blockade was verified by intradermal injection of methacholine. In each instance, experimental results were contrasted with a control site, i.e. the opposite palm or forearm.

Finally, fifteen healthy human volunteers (male and female) were given intravenous doses of either alpha- or beta-adrenergic blocking agents. Before and after adrenergic blockade, their spontaneous (base line) palmar sweating and their responses to intradermally injected adrenaline and to various emotional stimuli were recorded. Sweat determinations were performed on both palms, in order to separate intradermal and systemic effects. All measurements were recorded with the modified Bullard sudorometer.

The sudorometer directs air at ambient relative humidity into a plastic chamber (diameter 3·49 cm; height 2·54 cm) attached to the skin. Using lithium chloride sensors (Hydrodynamics Inc., Silver Spring, Maryland, U.S.A.,), the relative humidity of the air is recorded both before and after passage through the chamber. The air temperature and air flow are then measured, kept constant and the percent change in relative humidity calculated. This change in relative humidity correlates directly with sweat production on the skin.

The subjects, men and women with an age range of 23–54 years, were divided into three groups of five each. The first group received the alpha-adrenergic blocker, phentolamine (Rogitine) in intravenous doses of 5–10 mg; the second, the alpha-blocker, phenoxybenzamine (Dibenyline), in i.v. doses of 0.5 mg/kg; and the third, the beta-blocker, propranolol (Inderal) in doses of 1–3 mg i.v. Blood pressure and pulse were monitored throughout each study and pharmacological evidence of adrenergic blockade was provided by recording interference with the pressor and chronotrophic effects of intravenous adrenaline (10 μg), noradrenaline (10 μg) and isoproterenol (0.4 μg/kg/min).

All pharmacological agents employed in this study were diluted in phosphate-buffered physiological saline (pH 7·4) prior to use.

RESULTS

Palmar sweating, quantitatively recorded by the modified Bullard sudorometer, was noted to be cyclic, periodically rising above and falling below the mean base line level (Fig. 1). Sweat secretion on the palm was further observed to be exquisitely sensitive to emotional factors, rising in response to painful or stressful stimuli, such as saline injection or mental arithmetic, and returning to base line range with removal of the stimuli (Fig. 1).

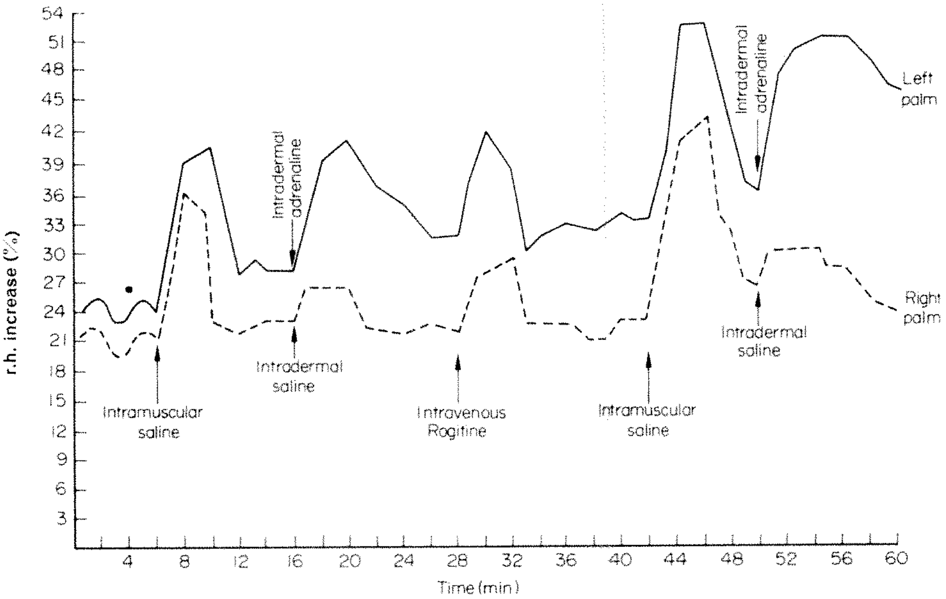

FIGURE 1. The effect of alpha- and beta-adrenergic blockade on palmar sweating. The increased sweating over base line following intramuscular and intradermal saline returns to base line on removal of stimulus. Note that phentolamine blockade (dashed line) has no effect on spontaneous, emotional or adrenergically-induced palmar sweating (similar results with phenoxybenzamine and propranolol). This pattern was seen with all fifteen subjects receiving adrenergic blocking agents.

Intravenous doses of phentolamine, phenoxybenzamine and propranolol inhibited the pressor and chronotrophic effects of intravenous adrenaline, noradrenaline and isoproterenol (Goodman & Gilman, 1965). In spite of this proven adrenergic blockade, however, there was no diminution of spontaneous palmar sweating (Table 1 and Fig. 1). Nor was there any evidence that either alpha- or beta-blockade altered the sudorific response to emotional stimulation or intradermal adrenaline (Table 1 and Fig. 1).

Of the twenty-three forearms injected with intradermal adrenaline, fifteen (65%) revealed a significant (>8% increase in relative humidity; range 8–52% increase) sudorific response contrasted with the opposite uninjected forearm. Thirty-six per cent (ten of twenty-eight) of the adrenaline-injected

TABLE 1. The effect of alpha- and beta-adrenergic blocking agents on palmar sweating, blood pressure and pulse rate (see Fig. 1)

Blocking agent	Spontaneous palmar sweating	Emotional palmar sweating	Adrenaline-induced palmar sweating	Acetylcholine-induced palmar sweating	Blood pressure	Pulse rate
Phentolamine (Rogitine)	No effect	No effect	No effect	No effect	Decrease	Increase
Phenoxybenzamine (Dibenyline)	No effect	No effect	No effect	No effect	Decrease	Increase
Propranolol (Inderal)	No effect	No effect	No effect	No effect	Decrease	Decrease

palms showed a similar positive response. A positive response to adrenaline begins within the first minute after injection, peaks at 3–5 min and lasts longer than 15 min (up to 1 h). In contrast, the response to intradermal saline begins almost immediately, but peaks in the first 2 min and rarely persists beyond 5 min. The effect of intradermal methacholine has a slower onset, peaks in 6–8 min and decreases gradually to base line over a period of 15–25 min (Gordon & Maibach, 1965) (Table 2).

TABLE 2. Sudorific response to intradermal injection of adrenaline, physiological saline and methacholine. (These data are summarized from observations of palms and forearms in fifty-one subjects studied.)

	Onset of action (min)	Peak effect (min)	Duration of effect (min)	Atropine effect	Lignocaine effect
Adrenaline	1	3–5	15–60	None	None
Saline	0·5	2	3–5	Inhibition of sweating	None
Methacholine	1–2	6–8	15–25	Inhibition of sweating	None

The methacholine effect is more reproducible and tends to stimulate a more dramatic rise from base line levels.

In all sixteen subjects studied for indirect release of acetylcholine or stimulation of the axon reflex, adrenaline-induced palmar and forearm sweating was not suppressed by either local anaesthesia or atropine blockade sufficient to inhibit the well-documented effects of methacholine (Table 2).

Of the fifteen palms monitored specifically for the effects of intradermal atropine blockade, emotional sweating was markedly suppressed in twelve instances. The level of spontaneous palmar sweating, however, was not lowered by atropine-induced cholinergic blockade (Fig. 2 and Table 3).

DISCUSSION AND CONCLUSIONS

It should be emphasized that the palmar eccrine gland exhibits at least three distinct types of sweating. The first is thermal sweating. The second, which we have called 'emotional' sweating, is that sweating provoked by anxiety or pain and is the pattern of sweat elaboration characteristically associated with the palm. In addition, however, there is a base line level of sweating. It is this continuous process, with cyclic elevation and diminution, that we refer to as 'spontaneous' palmar sweating.

Utilizing the modified Bullard sudorometer, we have confirmed earlier observations of the sudorific potential of intradermal adrenaline. Surprisingly, the sweat glands of the forearms were noted to be significantly more responsive to adrenaline ($P > 0·04$) than the palms. However, while we have demonstrated a marked difference in the sensitivity of palm and forearm, it seems obvious that the eccrine glands of both sites are capable of direct stimulation by adrenaline in some individuals. The persistence of this effect in the presence of verified anticholinergic blockade and clinically effective local anaesthesia would seem to exclude two previously postulated explanations of adrenaline-induced sweating, i.e. indirect release of cholinergic mediators and stimulation via the axon reflex (Table 2 and Fig. 2) (Wada, 1950; Sonnenschein *et al.*, 1951).

While both palm and forearm were generally sensitive to intradermal adrenaline, not all subjects responded to stimulation. In fact, only 36% of injected palms showed a positive sudorific response;

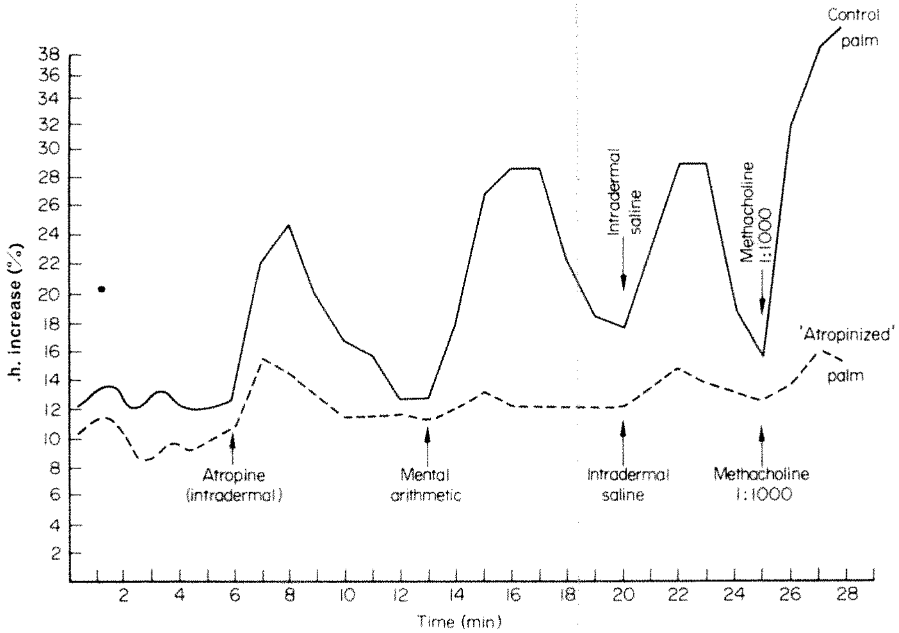

FIGURE 2. The effect of intradermal atropine blockade on spontaneous and emotional palmar sweating. Note that while atropine blockade (dashed line) produces almost complete suppression of emotional sweating (mental arithmetic, intradermal saline), there is no apparent decrease in spontaneous palmar sweating. Adequacy of atropine blockade is demonstrated by inhibition of methacholine-induced sweating in the 'atropinized' palm. This figure represents the response pattern of twelve of the fifteen palms studied. Positive responses (inhibition of mental sweating without alteration of spontaneous sweating) included both males and females with an age range of 19–58 years.

this pattern was remarkably consistent in that individual subjects tended to show repeatedly positive or negative responses with repeat injections. These observations imply that there may be two populations of ostensibly 'normal' subjects: one whose palmar eccrine sweat glands respond to local catecholamine stimulation, and one whose glands do not. We have not attempted to correlate these data

TABLE 3. The effect of cholinergic (atropine-induced) and adrenergic (alpha and beta) blockade on spontaneous palmar sweating and on sweating produced by emotional, adrenergic and cholinergic stimulation. (Each study involved fifteen subjects. Both sexes were included and ages ranged from 19 to 58 years. The data is summarized here and in succeeding tables in order to avoid presentation of lengthy statistical tabulations.)

	Atropine blockade	Alpha- or beta-adrenergic blockade
Spontaneous palmar sweating	No effect	No effect
Emotional palmar sweating	Inhibition	No effect
Methacholine-induced palmar sweating	Inhibition	No effect
Adrenaline-induced palmar sweating	No effect	No effect

with patterns of clinical disease, e.g. the atopic diathesis, or with response to other sudorific agents. Such studies would be of interest, clinically and physiologically, especially with regard to the well known pharmacological aberrancy of the atopic state.

In contrast to some earlier observations (Haimovici, 1950; Foster & Weiner, 1970), our current study reveals that spontaneous, emotional and adrenergically induced palmar sweating in man are not inhibited by alpha- or beta-adrenergic blocking agents in pharmacologically active doses (Fig. 1; Tables 1, 3 and 4). Similar studies in animal models, such as the ox (*Bos taurus*) (Findlay & Robertshaw, 1965) and the donkey (*Equus asinus*) (Bullard *et al.*, 1970; Robertshaw & Taylor, 1969), have also shown increased eccrine sweating with intradermal adrenaline and no change in sweat rate following alpha- or beta-adrenergic blockade.

TABLE 4. The effect of propranolol on palmar sweating, arterial pressure, and pulse rate. (These are mean values from the five subjects receiving propranolol. Similar data was recorded after alpha-adrenergic blockade.)

	Propanolol	
	Before	After*
'Spontaneous' sweating (base line)	40–48% Δr.h.	37–50% Δr.h.
Emotional sweating (rise from base line)	+28% Δr.h.	+30% Δr.h.
Adrenaline-induced sweating (rise from base line)	+31% Δr.h.	+35% Δr.h.
Arterial pressure (mmHg)	108/70	96/58
Pulse rate (beats/min)	68	54

* Following administration of the beta-blocker, propranolol, there was no increase or decrease in spontaneous (base line) palmar sweating, and, as with the alpha-blockers, no inhibition of emotional or adrenaline-induced sweating. The blood pressure fell, as did the pulse rate, a combination characteristically seen with beta-adrenergic blockade.

Our findings did not confirm those of Foster & Weiner (1970), who also utilized a quantitative measurement of sweating but produced inhibition of adrenergic sweating with high doses of locally injected phentolamine and guanethidine. The two studies differed, however, in anatomical region (palm v. forearm), type of sweating (emotional v. thermal) and route of administration of blocking agents (systemic v. local). Our study concentrated upon palmar eccrine sweating, which is very sensitive to emotional stimulation and quite distinct from thermally-induced forearm sweating. Foster & Weiner concluded, as have many others, that the mediation of eccrine sweating is entirely cholinergic. Yet, to our knowledge, no proponent of this theory has adequately explained the failure of atropine, locally or systemically, to block adrenaline-induced sweating. One must now also explain the failure of atropine blockade to produce suppression of base line palmar sweating.

Recently, Hemels (1970) has described an increased eccrine sweat gland response to intradermal acetylcholine following the intradermal injection of propranolol. Foster, Haspineall & Mollel (1971) found no marked propranolol influence on acetylcholine-induced sweating in man. In our studies, the systemic administration of propranolol (as with phenoxybenzamine and phentolamine) produced no significant alteration of eccrine sweating patterns at rest or in response to emotional stress, adrenaline or acetylcholine.

Our studies with intradermal atropine further reinforce the possibility that the classically conceived cholinergic chemical mediators may not alone be responsible for the complex physiology of palmar eccrine sweating. For, while atropine blockade was able to inhibit emotional sweating, it had no significant effect on the level of spontaneous palmar sweating (Tables 3 and 5 and Fig. 2). With cholinergic receptors inactivated by atropine and insensitive to methacholine stimulation, palmar sweating persisted at its base line rate, implying the existence of an additional (non-cholinergic) receptor. Thiele, Hemels & Malten (1972) have recently described compatible findings; they were unable to suppress completely the flow of water from human eccrine sweat glands using iontophoresis of atropine and scopolamine. They did not believe that the water loss seen after local atropinization is due to transepidermal water loss; they suggested that the areas of the palm rich in sweat glands may allow for special hydrodynamic water loss not related to sweating. Hence, an alternative explanation for the failure of atropine completely to suppress spontaneous palmar sweating might be that this fluid does not represent pharmacologically mediated eccrine sweat but rather non-pharmacologically mediated trans-eccrine water loss.

TABLE 5. The effect of cholinergic (atropine-induced) blockade on palmar sweating. (These are mean values of direct sudorometer recordings from fifteen subjects studied.)

	Atropine	
	Before	After*
'Spontaneous' palmar sweating (base line)	8–12% Δr.h.	11–14% Δr.h.
Emotional palmar sweating (rise from base line)	+13% Δr.h.	+2% Δr.h.
Methacholine-induced palmar sweating (rise from base line)	+24% Δr.h.	+4% Δr.h.
Adrenaline-induced palmar sweating (rise from base line)	+18% r.h.	+20% r.h.

* While emotional and methacholine-induced palmar sweating were inhibited by atropine blockade, there was no inhibition of adrenaline-induced sweating and no decrease of spontaneous (base line) sweating. Thus, in spite of complete cholinergic blockade, base line sweating remained stable.

The sudorific effects of intradermal adrenaline after cholinergic blockade thus imply that some adrenergic receptor site is important in the maintenance of base line (spontaneous) eccrine sweating. Alpha- and beta-adrenergic blockade had no effect on spontaneous or emotional palmar sweating, suggesting the possibility that a previously undescribed adrenergic receptor site is physiologically active in the mediation of eccrine sweating.

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Dithranol (anthralin) in psoriasis

THE EFFECT ON DNA SYNTHESIS, GRANULAR LAYER AND PARAKERATOSIS

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SUMMARY

Biopsies from affected skin of sixty-two patients with stable psoriasis were investigated by *in vitro* labelling with tritiated thymidine and by routine histological techniques.

In the same patient there were considerable variations in the number of DNA-synthesizing cells in biopsies taken from different but similarly located lesions of the same macroscopic appearance, whereas variations in the number of DNA-synthesizing cells in biopsies taken from the same lesions were less pronounced. With due regard given to this finding, it is shown that treatment with dithranol for 1 and 2 weeks significantly reduces the number of DNA-synthesizing cells. There was an inverse relationship comparing the development of the granular layer with the number of DNA-synthesizing cells and parakeratosis.

The variations in the number of DNA-synthesizing cells in different lesions in the same patient may also have implications for paired comparisons of topical treatment efficiency.

The mechanism by which dithranol exerts its therapeutic effect in psoriasis has been investigated in different ways in recent years; however, the findings of these studies have been controversial and no definite conclusion can be drawn.

In vitro investigations by Swanbeck & Thyresson (1965) have demonstrated an interaction between dithranol and DNA. An inhibitory effect has also been demonstrated on the regenerative capacity of amphibian larvae (Krebs & Schaltegger, 1965a) and on the number of synthesizing cells in the epithelium of guinea-pig skin (Swanbeck & Lidén, 1966). With respect to psoriatic lesions, however, the reports on the mode of action of dithranol are inconsistent. A decrease in mitotic counts after dithranol treatment has been reported several times (Krebs & Schaltegger, 1965b; Fry & McMinn, 1968; Baxter & Stoughton, 1970) and, in a recent study, Steigleder, Schumann & Lennartz (1973) found normalization of the shortened cell cycle with a decrease of the DNA synthesis. Born (1969), however, could not demonstrate any decrease in the number of DNA-synthesizing cells after dithranol in psoriasis. A good therapeutic response was supposed to follow a change from a parakeratotic to an orthokeratotic metabolism in the epidermis despite a continued high rate of proliferation of the epidermal cells. Fisher & Maibach (1971) found an increase in the mitotic counts of the epidermis of non-psoriatic skin after dithranol treatment. These disagreements between the different reports prompted this investigation, the purpose of which has been to study the effect of dithranol on the DNA synthesis in psoriatic lesions with due regard being paid to the pronounced variation in different lesions of the same patient which has been found in the present study.

MATERIAL AND METHODS

Sixty-two patients, thirty-three men and twenty-nine women between the ages of 13 and 80 years with stable psoriasis, were included in the study. They had not received any local treatment for at least 2 weeks prior to the start of the experiment, with the possible exception of 2% salicylic acid in white petrolatum. No changes in the internal medications were made during the investigation period.

Treatment

The psoriatic lesions were treated with either salicylic acid only (twice daily) or with both salicylic acid and dithranol (anthralin; Cignolin, Bayer, West Germany). Salicylic acid was applied in 2% strength and the dithranol in 0·5% strength, both in white petrolatum. No dithranol ointments were used which were older than 1 month at the time the experiment was begun. If there was pronounced scaling, treatment with salicylic acid was given twice daily for 3 days before beginning the treatment with dithranol. Patients treated with dithranol had one application of dithranol each day, either in the morning or in the afternoon, and the salicylic acid ointment was applied at the alternate time of day. If dithranol caused irritation of the skin, it was temporarily replaced by the salicylic acid preparation.

Biopsies

Punch biopsies, 3 mm in diameter, were taken without local anaesthesia between 8 a.m. and noon. The biopsies were divided into two equal parts by a cut perpendicular to the skin surface with a razor blade. Two biopsies were taken from most of the patients but three or more were taken from some of them. A total of 160 biopsies were examined. The lesions selected for study were homogenous, nummular or geographic plaques, mostly on the lower back, buttocks or thighs. Friction areas overlying bony prominences were avoided. The biopsies were taken according to the following scheme:

(a) Two biopsies at the same time from one untreated psoriatic plaque. The biopsies were taken at least 2 cm apart at the same distance from the periphery of the lesion.

(b) Two biopsies at the same time from different untreated psoriatic plaques having the same appearance and from the same part of the body in the same patient.

(c) One biopsy before and one after treatment of the plaque with 2% salicylic acid in white petrolatum.

(d) One biopsy before and one or more biopsies after treatment of the plaque with dithranol in white petrolatum either once only or once daily for 1 or 2 weeks, and at different intervals following the only or last application of dithranol. Salicylic acid ointment was given during the same treatment period.

Labelling

In vitro labelling of the biopsies was performed in a culture medium consisting of Parker 199 containing 1 μCi of tritiated thymidine per ml (specific activity 6·7 Ci/mmol, New England Nuclear Corp., Boston, Mass., U.S.A.). The radioactive culture medium was stored in sterile vials at +4°C for no more than 14 days. Within 5 min of taking the biopsy both parts of it were placed in a sterile test tube containing 5 ml of the culture medium which had been prewarmed to 37°C. The test tubes containing the biopsies were incubated for 2 h in a 37°C waterbath. No special gaseous aeration was used. After 1 h the test tubes were shaken thoroughly. Continuous agitation was not employed. The specimens were washed and fixed according to Lachapelle & Gillman (1969). The cut surface created by the razor blade was gently pressed against a coverslip before being placed in the fixative in order to retain its shape.

After routine histological processing, 5 μm sections were cut. Care was taken to orient the paraffin block so as to obtain sections of the entire epidermal part of the flat-cut surface. Beginning with the very first section, all sections were mounted on gelatin-coated slides.

The slides were coated with Kodak NTB-2 liquid emulsion according to routine methods. They were placed in airtight boxes containing silica gel at +4°C for 2 weeks. After developing in Kodak D-19 for 2 min, rinsing and fixation in Kodak F-24, the slides were stained through the emulsion with Mayer's alum haematoxylin and mounted.

Evaluation of the autoradiographs

The autoradiographs were examined at a magnification of ×320 by an observer who was unaware of the design of the experiment. As a rule, fifteen such high power fields were screened, corresponding to a surface length of 4·4 mm. The first serial section of good technical quality was screened and then another one or two sections if necessary. In order to avoid repeated counting of the same cells, at least three sections were skipped between those selected for counting. The epidermal cells incorporating tritiated thymidine were, as a rule, heavily labelled. Only very occasionally was there any doubt whether a cell was labelled. In these instances an arbitrary limit of five grains over the nucleus was chosen as a criterion of true labelling.

The first 100 biopsies were evaluated in two ways. The number of labelled cells in the three basal rows of epidermal cells was correlated both with unit surface length and with unit basal membrane length. Analysis of regression of these two series of data showed a very good correlation ($r = +0{\cdot}91$). Hence, the less time-consuming method of counting labelled cells per unit surface length was adopted, one unit surface length being defined as one high power field (×320), corresponding to 0·3 mm. Thus the absolute number of labelled cells per mm surface length can be obtained by multiplying the figures given in the tables by a factor of 3·3.

The mean number of labelled epidermal cells per unit surface length (labelling frequency) in the biopsy taken before starting treatment with dithranol was defined as 100%. The counts in the biopsies taken after treatment were related to this initial control value in terms of per cent (relative labelling frequency).

Evaluation of granular layer and parakeratosis

All the biopsies taken 24 h after the last dithranol application in each of the three treatment groups (single application, 1 and 2 weeks' application) were included in this part of the study.

Sections were cut from the same half of the biopsy as that used for the autoradiography sections. These sections were stained with Haematoxylin and Eosin. The degree of parakeratosis, as well as the development of the granular layer, was assessed by two persons independently. A homogeneous parakeratosis, evenly distributed in the entire basal portion of the horny layer, was designated as + + +, while a patchy and sparse parakeratosis was marked +, and the intermediate state indicated by + +. For the granular layer, + + + indicates normal appearance and + a sparse and irregular development.

RESULTS

The labelling frequency, measured as the number of labelled cells per unit surface length, in two biopsies taken from the same psoriatic lesion at the same time is given in Table 1. The variation between the counts from the two biopsies in each patient has been determined by using the formula

TABLE 1. Comparison of the labelling frequencies of epidermis (number of DNA-synthesizing cells per unit surface length) in biopsies from nine patients taken at the same time from the same psoriatic lesion. S is a measure of the variation between the two counts in each pair of data

Patient no.	No. of labelled cells/unit surface length	
	Biopsy 1	Biopsy 2
1	9·9	9·4
2	19·0	19·3
3	13·1	15·0
4	24·9	26·8
5	23·3	19·5
6	27·9	30·0
7	13·1	12·2
8	38·9	44·3
9	31·4	43·1
	S = 3·27	

$S = \sqrt{(\Sigma d_i^2)/2n}$, where n = number of paired determinations and d_i = difference between the two determinations in each pair. The variation thus determined is 3·27.

The labelling frequency in two psoriatic plaques of the same appearance and located on the same part of the body in the same patient is shown in Table 2. There were considerable differences in the number of labelled cells. In a few instances the differences exceeded 100%. The variation between the

TABLE 2. Comparison of the labelling frequencies of epidermis in biopsies from thirteen patients taken at the same time from different psoriatic lesions of the same macroscopic appearance. The variation between the two counts is significantly greater for the values in Table 2 than in Table 1 ($P < 0·001$)

Patient no.	No. of labelled cells/unit surface length	
	Plaque 1	Plaque 2
10	20·1	14·0
11	24·7	13·1
12	13·6	23·5
13	24·3	19·3
14	26·7	27·9
15	17·6	22·5
16	25·7	59·9
17	41·5	18·9
18	23·7	34·0
19	13·4	23·7
20	26·2	19·0
21	26·3	31·5
22	46·7	22·4
	S = 10·54	

values in this situation was much larger than when the biopsies were taken from the same lesion ($S = 10{\cdot}54$ and $3{\cdot}27$ respectively, $P < 0{\cdot}001$).

After application of salicylic acid to psoriatic lesions, seven of nine treated patients had a slight, but probably insignificant, increase in the labelling frequency, as shown in Table 3.

TABLE 3. Effect of external applications of 2% salicylic acid in white petrolatum on the labelling frequency in epidermis from psoriatic lesions

Patient no.	No. of labelled cells/unit surface length		
	Before treatment	After treatment	No. of days
23	16·0	18·7	3
24	10·8	10·9	3
25	21·4	32·7	3
26	53·5	46·1	3
27	24·3	32·7	3
28	26·1	28·1	4
29	9·7	12·5	7
30	20·7	19·1	7
31	17·3	22·2	7

The effect of dithranol treatment is shown in Fig. 1. After a single application (Fig. 1a), a wide scattering of the relative counts was observed with decreased as well as increased counts. This scattering was present regardless of the interval between application of dithranol and taking the biopsy. No conclusions can therefore be drawn concerning the effect of a single application of dithranol.

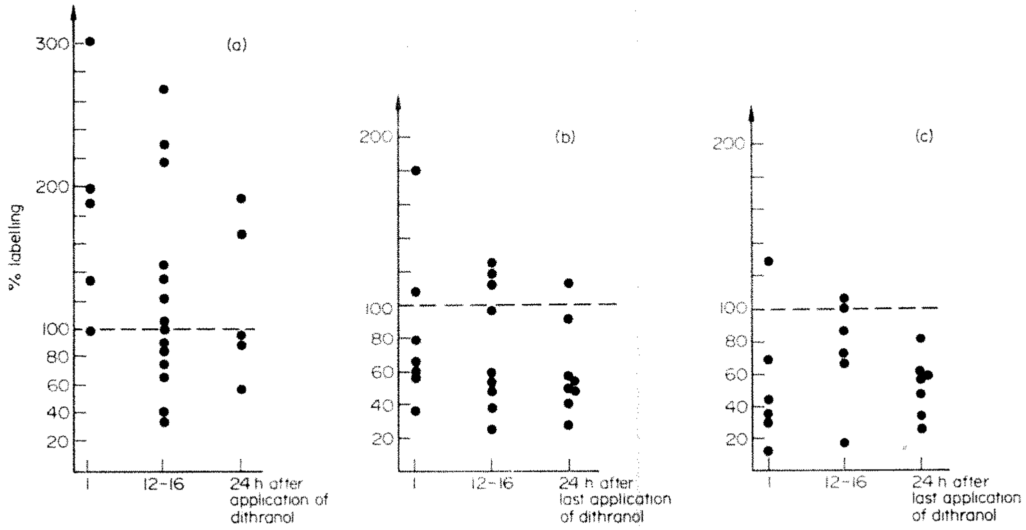

FIGURE 1. Effects of treatment with dithranol on the relative labelling frequencies of epidermal cells in psoriatic plaques (application once daily). The value of the labelling frequency before treatment = 100%. (a) One single application; (b) treatment during 1 week—decrease ($P < 0{\cdot}025$); (c) treatment during 2 weeks—decrease ($P < 0{\cdot}005$).

After treatment for 1 week (Fig. 1b), there was a decrease in labelling frequency. This decrease was observed in biopsies taken at all three intervals following the final application of dithranol. The results after treatment for 2 weeks (Fig. 1c) were comparable to the results after 1 week of treatment but the decrease in labelling frequency was more pronounced.

As there were no clear-cut differences in labelling frequency between the biopsies taken 1, 12–16, or 24 h after the final application of dithranol, all the results after each treatment period (single, 1-week, and 2-week) have been pooled. By use of the sign test, a statistically significant decrease in labelling frequency after dithranol treatment can be demonstrated after 1 week ($P<0{\cdot}025$) and after 2 weeks ($P<0{\cdot}005$) compared to before treatment.

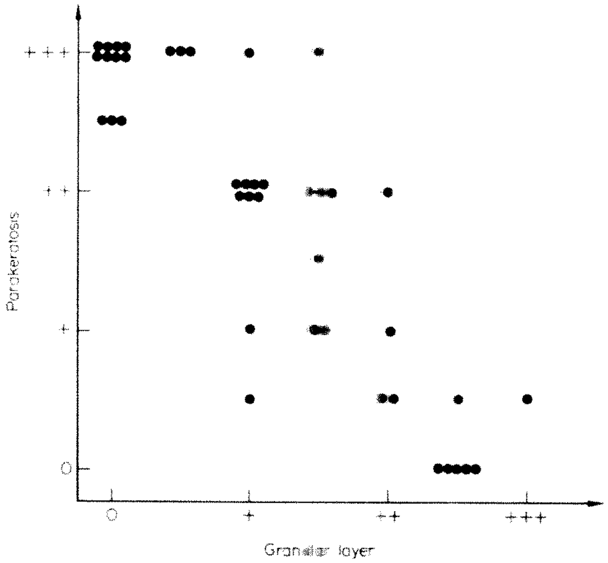

FIGURE 2. Relationship between presence of parakeratosis and the state of the granular layer in untreated and treated psoriatic plaques. Both these parameters were graded arbitrarily between absence (0) and full development (+ + +). All the biopsies taken before treatment and 24 h after the only or last application of dithranol to the same lesions are included.

The relationship between the presence of parakeratosis and the development of the granular layer is shown in Fig. 2. An obvious tendency to an inverse relationship between these two parameters was observed. A χ^2-test confirmed this observation ($P<0{\cdot}001$).

The same semi-quantitative results from the assessments of the granular layer have been plotted against the relative labelling frequency (initial value = 100%) in Fig. 3. A decreasing labelling frequency thus corresponds to an increasing development of the granular layer.

DISCUSSION

Methodological aspects

In previous work involving *in vitro* labelling of skin (Born, 1968; Lachapelle & Gillman, 1969), thin slices were cut to facilitate the penetration by the radioactive marker. For correct counting it is desirable that the cuts be made perpendicularly to the skin surface. Therefore, the biopsies in this experiment were cut only once, along one diameter, as this seemed to facilitate the orientation of the cut.

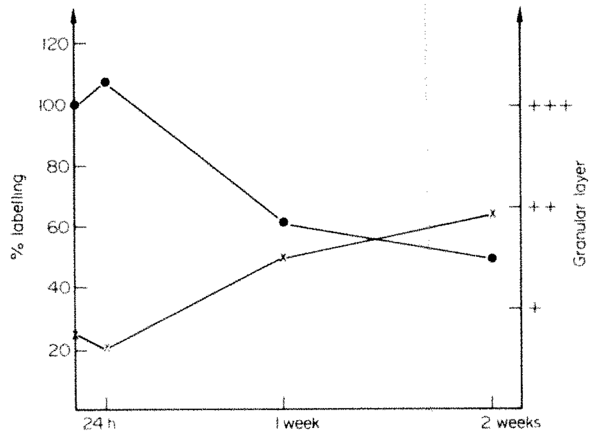

FIGURE 3. Relationship between the relative labelling frequencies of the epidermal cells and the presence of granular layer in psoriatic plaques treated with dithranol for various periods of time. The lines connect the mean values of relative labelling frequencies and granular layer development respectively of the same biopsies as those used for preparing Fig. 2. Each psoriatic lesion has delivered one biospy before treatment (labelling frequency = 100%) and one or more biopsies after treatment. ●—●, indicates relative labelling frequency; ×—×, indicates granular layer development.

The penetration of the marker is sufficient for reliable counting provided the sections are collected and mounted from the very first one. The two to three sections required for counting can then be sampled from the first ten to twenty serial sections. The correlation between labelling indices obtained after labelling *in vivo* and *in vitro* has been investigated by several authors and with partly conflicting results (Helpap & Maurer, 1969; Denekamp & Kallman, 1973). In the present work, the pertinent fact is that all the biopsies have been handled in a uniform way. Even if the obtained values deviate from the true *in vivo* conditions, they thus ought to be mutually comparable provided the utilization of the marker by the epidermal cells does not change during the process of healing.

The measurement used in this study to determine the number of cells in the S-phase at the time of labelling is called the labelling frequency. The labelling index, i.e. per cent labelled cells, is highly dependent on the total number of cells in the epidermis, i.e. on the degree of acanthosis. The labelling frequency, on the other hand, takes into account the number of labelled cells per unit length, either of the basal membrane or of the surface. In this way the growth fraction of the epidermal cells is evaluated regardless of the varying number of cells not belonging to this fraction.

Theoretically, counting of the labelled cells in the basal rows of the epithelium ought to give the most reliable results as this method compensates for the pronounced differences in basal membrane length between different psoriatic lesions. Inasmuch as the correlation between the counts per unit basal membrane length and per unit surface length was very good ($r = +0{\cdot}91$), the latter measure was chosen on the merits of greater technical simplicity.

The number of cells in S-phase at a given moment also depends on the length of this part of the cell cycle. According to Weinstein & Frost (1971), the S-phase in psoriatic epidermal cells is shorter than in normal epidermal cells (8·5 and 16 h respectively). No figures for psoriatic lesions in different stages of healing are available. If the duration of the S-phase increases during the process of healing, as it seems reasonable to assume, the number of labelled cells should increase provided the fraction of DNA-synthesizing cells remains the same. In other words, the possible prolongation of the S-phase tends to obscure a diminution of the DNA-synthesizing cell pool and, thus, the figures of labelled cells after treatment presented in this work must be considered as minimum figures.

The pronounced variations between different psoriatic lesions of the same appearance in the same patient are noteworthy. It must be emphasized that the experimental conditions were absolutely identical for each pair of biopsies. They were taken simultaneously and handled parallel to each other in every step of the experimental procedure. Comparisons of the mitotic counts in different plaques in the same patient have previously been reported by Fry & McMinn (1970) and Cox & Watson (1972). Fry & McMinn came to the conclusion that the variation between different lesions in the same patient is insignificant compared to the variation found when lesions from different patients are compared. They give no figures for the individual lesions and comparisons with our results are, therefore, not possible. In the work of Cox & Watson however, the differences between two plaques from the same patient are considerable in many cases. Calculated on a percentage basis the differences are of nearly the same order of magnitude as in the present work. Thus, the inter-lesional variation may be remarkably large, a fact which has obvious implications for paired comparisons of topical treatment.

In order to obtain an optimal effect from external application of dithranol, penetration of the substance must not be retarded or stopped by the psoriatic scales. In this experiment keratolysis was accomplished by the use of salicylic acid in white petrolatum. The finding that treatment with this preparation tends, if anything, to increase the labelling frequency is in accordance with the mitosis-stimulating effect of pure petrolatum in guinea-pig skin (Born, 1968). The observed differences were so small, however, that an effect on the outcome of the dithranol treatment seems unlikely.

Effect of dithranol treatment

According to the present work, treatment with dithranol decreases the labelling frequency in the psoriatic epidermis as compared with that before treatment. This finding is considered to reflect a decrease in the total epidermal DNA synthesis according to arguments presented earlier in this article. The decrease in labelling frequency is not apparent after 24 h. At that stage of treatment, the stimulating effect of dithranol on DNA synthesis ('acanthogenic effect', see Born, 1969) seems to be at least as pronounced as its depressing effect.

The decrease is clearly discernible after 1 week and still more evident at 2 weeks. These results confirm the mitotic count investigations published by Krebs & Schaltegger (1965b), Fry & McMinn (1968) and Baxter & Stoughton (1970). They are, however, in disagreement both with the mitotic count findings of Fisher & Maibach (1971) after dithranol on normal skin, and those reported by Born (1969) in his study of the dithranol effect in psoriasis where *in vitro* labelling of cells in DNA synthesis was used. The lack of response found by Born might be explained by inter-lesional variation (no statement is made regarding the site of biopsy) and by inter-individual variation, which has been shown to be even greater than the inter-lesional variation (Fry & McMinn, 1970; Cox & Watson, 1972). If no attention is paid to these factors, differences between the labelling frequencies could easily be masked unless the number of biopsies is very large. Fisher & Maibach (1971) tested the effect of dithranol on epidermal mitotic activity in healthy (not psoriatic) subjects. It might be speculated that dithranol has a predominantly acanthogenic effect on normal skin as they found a definite increase in mitotic activity. Such an effect from dithranol has also been observed during the present investigation in patients with unstable psoriasis. In further studies on hairless mice, Fisher and Maibach found that the mitotic activity after dithranol treatment varied in a cyclic manner. No comment was made on the relation of this finding to the diurnal variation in mitotic activity in both man and animals. If this cyclic activity after dithranol is explained by another factor than the diurnal variation, it does not seem to work in the same way in man, as we found no differences in DNA synthesis between the biopsies taken 1, 12–16, or 24 h after dithranol application and at the same time of day throughout.

The question of whether the inhibition of the DNA synthesis is the primary event or is secondary to other changes cannot be answered by this investigation. Twenty-four hours after treatment with dithranol, no consistent changes had taken place either in labelling frequency, parakeratosis, or in the development of the granular layer. On the other hand, at the time of the next biopsy after 1 week's treatment, changes in all of those variables could be registered. In several previous studies, the reappearance of the granular layer has been found to precede a 'significant' decrease in the mitotic counts (Theune & Wohlrab, 1966; Fry & McMinn, 1968; Cox & Watson, 1972). Also, the first detectable changes in the enzyme-activity pattern after dithranol treatment have been reported to start in the upper layers of the viable epidermis and not basally (Braun-Falco, Burg & Schoefinius, 1971).

In some investigations, a normalization of the mitotic rates to those of non-affected psoriatic skin has been adopted as the definition of decreased mitotic activity (Fry & McMinn, 1968; Cox & Watson, 1972). It seems reasonable, however, to assume that even smaller decreases in mitotic activity could be of value for the healing of psoriatic lesions by reducing the acanthotic process. The alterations in enzyme activity which are detectable after a few days of dithranol treatment are not followed by an orthokeratosis, i.e. complete disappearance of the parakeratosis, until 3 weeks or more after the start of the treatment (Braun-Falco *et al.*, 1971). These authors therefore posed the question to what extent the rapid dithranol effect on the enzymes really normalizes the differentiation process of the epidermal cells.

The inverse relationship between the degree of parakeratosis and the development of the granular layer is in accordance with the recently reported results of Cox & Watson (1972). The correlation between a decrease in labelling frequency with the reappearance of the granular layer, as illustrated in Fig. 3, seems to differ from the results obtained by Fry & McMinn (1968) and Cox & Watson (1972). The discrepancy between our results and the results presented in those two papers could be explained by differences in the methods used. These latter studies correlate one mitotic-count value per individual with the state of the granular layer; however, the variation in the mitotic counts between lesions from different individuals is pronounced. Therefore, a correlation between such absolute mitotic count values and the development of the granular layer could not be expected. In our experimental model, the changes in labelling frequency and in the granular layer development are compared within one single lesion at different times, i.e. the inter-individual and even inter-lesional variations are eliminated. In this way the concomitant changes in DNA synthesis and the keratinization process can be demonstrated.

The results of the present investigation indicate that dithranol treatment decreases the number of DNA-synthesizing cells in the psoriatic epidermis. This decrease is correlated with an improved development of the granular layer. The design of the experiment does not permit an analysis of the relationship in time between these two changes, but previous reports suggest that the granular layer alterations precede the lowering in DNA synthesis. It is not clear, however, whether these two events are interdependent.

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Treatment of vitiligo with 0·1% betamethasone 17-valerate in isopropyl alcohol—a double-blind trial

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SUMMARY

Nineteen patients with symmetrically distributed patches of vitiligo took part in a double-blind trial comparing 0·1% betamethasone valerate in 50% isopropyl alcohol with the unmedicated base. Fifteen patients responded to the medicated application, none to the base alone. Two patients failed to respond and two patients defaulted from the trial.

Although complete spontaneous repigmentation is quite unusual in vitiligo (El-Mofty, 1968; Rook, Wilkinson & Ebling, 1972) and repigmentation has followed intradermal injections of some patches of vitiligo with triamcinolone acetonide (Kandil, 1970a), it was felt that for proper evaluation of the therapeutic role of betamethasone 17-valerate (BV) in vitiligo, a double-blind trial was indispensible.

Since there was no appreciable decrease in the time required to achieve considerable repigmentation or complete cure in cases treated topically with 0·2% BV in flexible collodion (FC) as compared to those treated with intradermal injections of triamcinolone acetonide (Kandil, 1970a), it was thought that a concentration of 0·1% BV might also be effective and perhaps more suitable. Because BV is more readily available from a 50% isopropyl alcoholic base than from flexible collodion (Wilson, 1972), it was decided to use 0·1% w/w BV in isopropyl alcohol: (BV-A) (Betnovate scalp application) and the unmedicated base as a control for this study.

MATERIALS AND METHODS

In a preliminary trial three patients with symmetrically distributed lesions of vitiligo were treated with either 0·1% betamethasone valerate in alcohol or 0·2% betamethasone valerate in flexible collodion applied twice daily. After the encouraging results of the preliminary trial, nineteen patients were selected with vitiliginous patches which were as much as possible comparable in site, size and duration. For each patient two types of application were supplied, one containing 0·1% betamethasone valerate in 50% isopropyl alcohol and the other containing only the alcohol base. The medicated and unmedicated applications were randomly allocated to the right and left patches and were applied twice daily. Neither the investigator nor the patients were aware which of the two applications contained the active principle. While fourteen cases have had their treated vitiliginous patches on the exposed parts of the skin, five patients had them on unexposed areas.

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RESULTS

The results of the short preliminary trial showed that 0·1% betamethasone valerate in alcohol was as effective as 0·2% betamethasone valerate in flexible collodion (Table 1). See also Plates 1 and 2.

TABLE 1. Results of preliminary trial

Case no.	Age (years)	Duration	Site	Left side	Right side	Results
1	25	2 months	Chin	0·1% BV-A	0·2% BV-FC	Complete cure of both sides within 3 months
2	12	3 months	Face	0·1% BV-A	Untreated	Complete cure of left side only within 4 months
					0·1% BV-A*	Complete cure within 4 months
3	24	2 years	Ankles	0·1% BV-A	0·2% BV-FC	No response on both sides within 3 months

* Treatment of the right side of the face was initiated only after complete cure of the left side, i.e. after acting as a control.

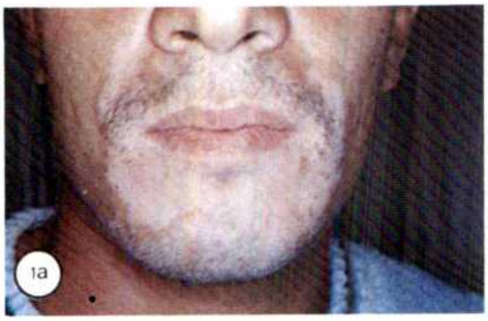
In the double-blind trial none of the nineteen patients showed any clinically noticeable repigmentation on the side to which the unmedicated base was applied. Fifteen cases were cured or improved on the sides to which the betamethasone valerate was applied (Plates 1 and 2). Of the seventeen patients who completed 4 months treatment, only two failed to respond to the medicated application. Two patients were lost trace of some time after starting the trial. Complications of treatment with the betamethasone valerate were limited to hypertrichosis in two patients and a localized acneiform eruption in three other cases.

PLATE 1. Case 1. (1a) Perioral vitiligo before treatment (23 October 1971). (1b) Equal response to topical applications of 0·2% BV-FC and 0·1% BV-A for 3 months (to the right and left parts respectively).

PLATE 1. Case 2. (2a) Vitiligo of the left side of the face before treatment (25 November 1971). (2b) Considerable repigmentation on 19 January 1972. (2c) Complete repigmentation after 10 weeks' treatment (8 March 1972).

PLATE 1. Case 2. (3a) Right side of the face kept untreated as a control for the same period (25 November 1971). (3b) Negligible improvement (patient admitted applying the treatment to the control side as well for some days after noting the result of treatment of the left side—8 March 1972).

Plate 1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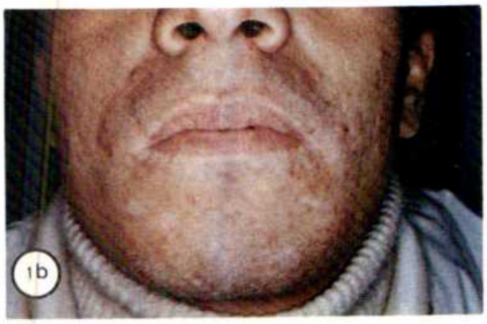
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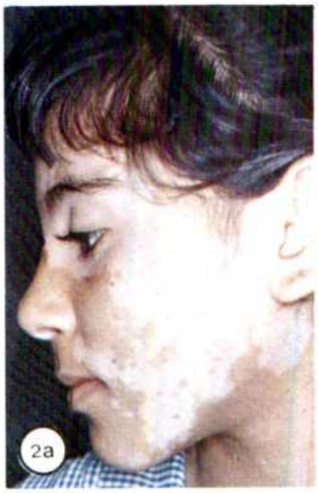
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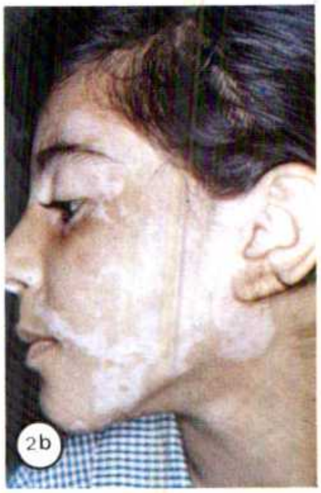
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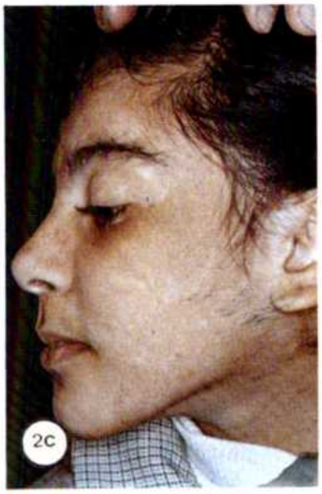
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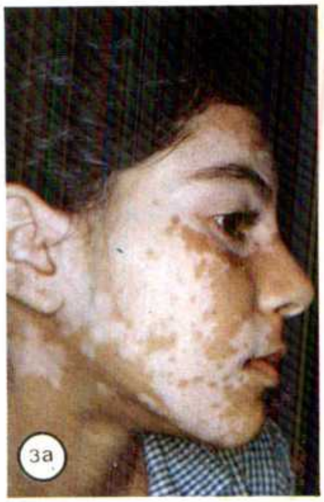
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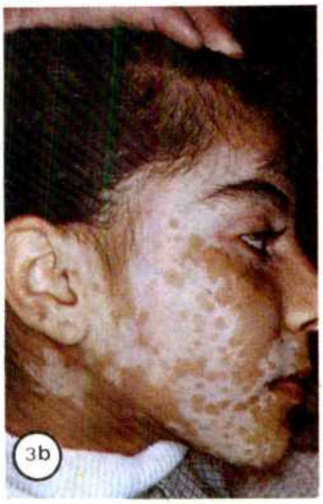
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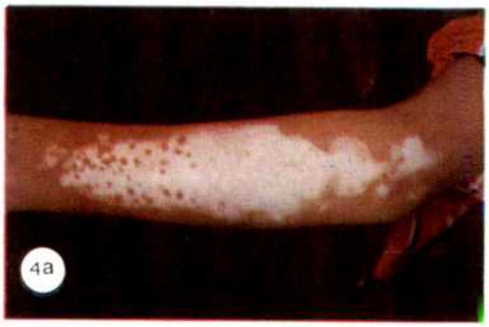
Plate 2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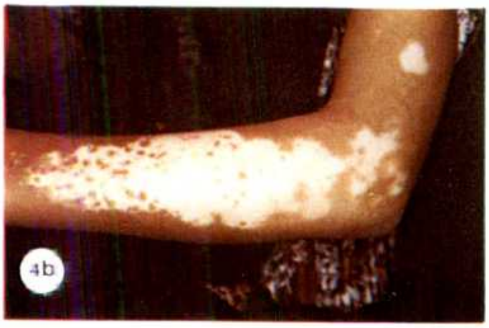
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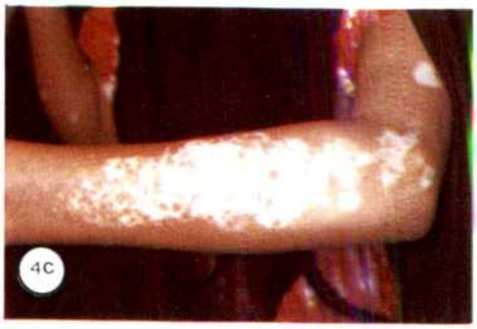
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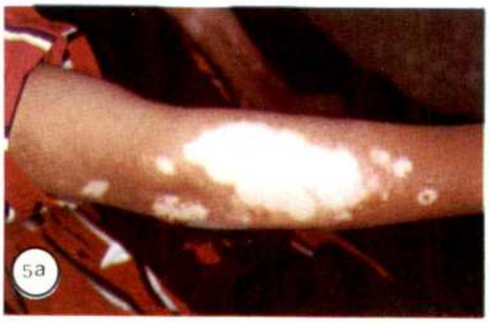
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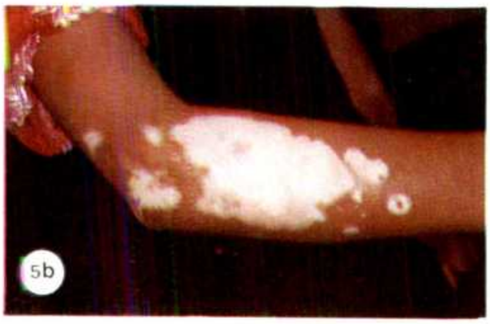
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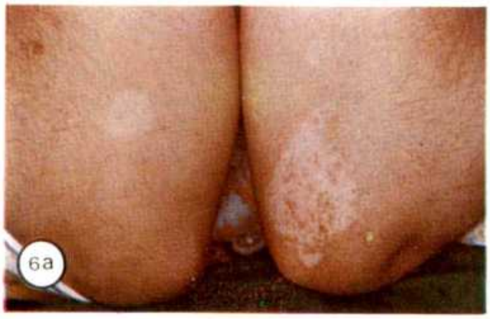
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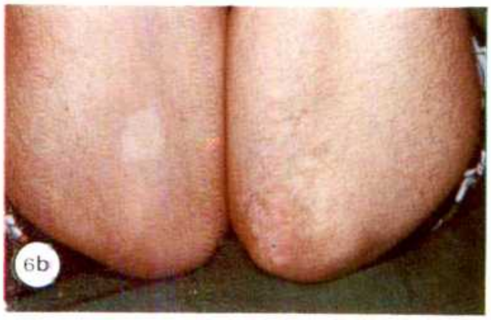
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TABLE 2. Comparison of treatment with 0·1% BV-A and unmedicated base

Clinical progress	No. of patients
Repigmentation or complete cure with 0·1% BV-A	15
Repigmentation with unmedicated base	0
No repigmentation	2
Lost to follow up	2
Total	19

TABLE 3. Results after 4 months' treatment with 0·1% BV-A

Clinical progress	No. of patients
Complete cure (90-100%)	6 (9 patches)
Good repigmentation (25-90%)	3
Beginning repigmentation	6
No response	2
Lost to follow up	2
Total	19

DISCUSSION

Until recently there was no alternative effective treatment for vitiligo patients not responding to psoralen therapy. The results obtained in this double-blind trial, and in previous reports (Kandil, 1970b; Koopmans-van Dorp *et al.*, 1973), show that betamethasone valerate is useful in the treatment of vitiligo despite some apparent contradictory experience of other authors. Koopmans-van Dorp *et al.* (1973) have used 0·2% betamethasone valerate in a DMSO cream base with exceptionally good results. On the other hand, Shelley (1972) using 0·2% fluocinolone acetonide cream (Synalar forte) under Saran tape commented that 'at present, the best we can offer is little. Extreme patience is required and hence for many a cosmetic cover is far more suitable'.

The results of the present trial suggest that a regular, rather than a high corticosteroid supply is necessary for promotion of repigmentation. Further studies are, however, necessary to find out the lowest corticosteroid concentration capable of giving maximum therapeutic effect with minimum complications, particularly in view of the recent awareness of possible side-effects of stronger steroids used on the face. Further studies will also be necessary to establish whether repigmentation occurs as readily in fair skins compared with the rather darker skinned patients involved in this study.

PLATE 2. Case 8. (4a) Left forearm before treatment (25 February 1972). (4b) Beginning repigmentation after 4 months of treatment with medicated base (10 June 1972). (4c) Considerable repigmentation noted after 8 months' treatment (10 October 1972).

PLATE 2. Case 8. (5a) Right forearm before treatment (25 February 1972). (5b) Right forearm after 8 months' treatment with unmedicated base showing no response (10 October 1972).

PLATE 2. Case 7. (6a) Vitiligo of the elbows before treatment (13 July 1971). (6b) Improvement of the side treated with the medicated base only (2 September 1971).

ACKNOWLEDGMENTS

I am greatly indebted to Dr G.Roux, Director of the Medical Department of Glaxo International, for the technical assistance offered by him, and for covering the cost of the colour plates, and to Mr R.T.Garrett for preparing, coding and later decoding the materials for the double-blind trial.

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Case Reports

Keratoacanthoma and multiple carcinomas

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SUMMARY

A case of keratoacanthoma, preceding four primary malignant tumours without metastasis, is presented. Two similar cases from the literature with two and three multiple primary carcinomas, are reviewed. They all have the following findings in common: (1) keratoacanthoma, (2) adenocarcinoma of the colon, (3) additional primary malignant tumour or tumours, and (4) absent or delayed metastasis.

In 1932 Warren & Gates established strict criteria for the pathological diagnosis of multiple primary malignancies. Fewer than thirty cases of four primary, histologically malignant tumours, occurring in the same patient, have been described to date in the world literature. Detailed study of the previous literature revealed no cases of keratoacanthoma of the face associated with four histologically distinct, primary, malignant tumours.

CASE REPORT

The patient, a 68-year-old white male, a clerk by profession, entered the Hampton, Virginia, Veterans Administration Hospital in May 1963. At that time the right side of his nose presented a raised nodular lesion measuring 8 mm in diameter (Fig. 1). The borders were sharply marginated and the centre was filled with warty material. The nodule was freely movable and there was no lymphadenopathy. The lesion had been present for 2 weeks.

Microscopic examination revealed central invagination of the squamous epithelium. The wall of the lesion was tunnelled. There was budding of squamous cells into the stroma at the base of the lesion but these cells lacked dysplasia. The diagnosis of keratoacanthoma was made.

In December 1963, the patient complained of four raised, brownish, somewhat crusted lesions, varying in size from about 1 to 1·5 cm in diameter, on his forehead. The lesions were superficial and not indurated. Seborrhoeic keratoses were diagnosed by the dermatologist. No biopsies were taken.

Four years later, the patient developed a large lesion on the left side of the lower lip, measuring 2 × 1·5 cm, deeply infiltrating the surrounding tissue. The central area was ulcerated and necrotic. The biopsy revealed squamous cell carcinoma. The patient received local X-ray therapy of 3200 rads total tumour dose. Although cancer of the lip is related to actinic radiation, there was no history of excessive sun exposure. The family history was non-contributory. The patient had no known history

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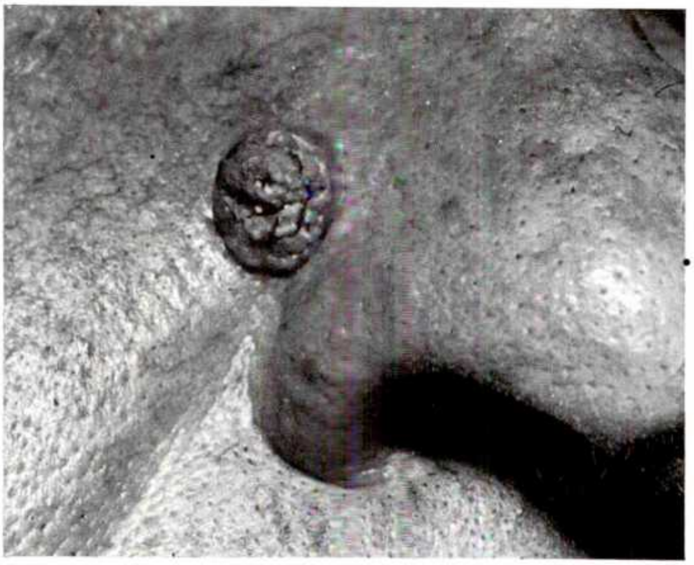

FIGURE 1. Keratoacanthoma—sharply marginated nodular lesion of the skin of the nose.

of syphilis, but he had one of heavy drinking. There was no evidence of exposure to arsenicals, toxic chemicals or immunosuppressive therapy.

His last admission was in 1970, at which time the clinical diagnosis of lower oesophageal malignancy was made. Autopsy revealed a squamous cell carcinoma at the oesophago-gastric junction, measuring 6 cm in diameter. The large bowel showed a well differentiated papillary adenocarcinoma of the ascending colon measuring 3 cm across. No polypi were present. In the prostate there was a moderately well differentiated adenocarcinoma.

The patient was free of metastases. Additional findings were: old myocardial infarction, emphysema and bronchopneumonia. There was no evidence of neoplasia at the site of the irradiated lip.

DISCUSSION

Keratoacanthoma usually occurs as a single lesion. However, multiple disseminated keratoacanthomas were reported by Winkelmann & Brown (1968) in association with polyposis of the large bowel but without internal organ malignancies.

Some investigators believe that the clinical course of keratoacanthoma can be explained on the basis of viral aetiology. Prieto *et al.* (1964) have produced strong experimental evidence in favour of a viral origin. Lowry, Clark & Hannemann (1972) suggested that perhaps keratoacanthoma and squamous cell carcinoma are the same disease in a different host. Patients with a good defence mechanism reject the neoplasm, whereas others whose resistance is impaired by disease or immunosuppressive agents are prone to malignant transformation of the keratoacanthoma.

In 1967, Muir, Yates-Bell & Barlow reported the case of a 44-year-old Maltese male with the following multiple consecutive neoplasms: keratoacanthoma of the face, squamous cell carcinoma of the larynx, adenocarcinoma of the colon, and adenocarcinoma of the duodenum. The tumours were surgically removed. There was no evidence of metastases found up to 2 years following the last surgical treatment.

Recently, Bakkar & Tjon (1971) described a 43-year-old male with both keratoacanthoma (right cheek) and sebaceous adenoma of the face, followed by adenocarcinoma of the ascending colon and, later, carcinoma of the transverse colon. A large adenocarcinoma of the stomach was subsequently excised. There was no evidence of metastasis 2 years later.

There is a resemblance between the current and the two previously published cases of Muir *et al.* (1967) and Bakker & Tjon (1971). Keratoacanthoma of the face was the initial tumour. Adenocarcinoma of the gastrointestinal tract occurred later. Despite the presence of multiple primary tumours of the internal organs, metastases were not found.

Analysis of all three cases points to several prominent facts. Firstly, all patients were male. Secondly, in all cases, keratoacanthoma of the face was the initial lesion. Thirdly, all three cases developed malignancy of the large bowel. Fourthly, all three cases had additional distinct malignant tumours. Fifthly, there was no evidence of metastasis from any tumour. The time interval between the diagnosis of keratoacanthoma and recognition of the subsequent malignancy varied between 1 and 6 years.

The characteristics common to all three cases suggest a related constellation of findings and possible syndrome: (1) facial keratoacanthoma followed by (2) adenocarcinoma of the colon, plus (3) additional distinct primary tumour or tumours and (4) absent or delayed metastasis.

Sommers & McManus (1953) reported three cases of arsenical skin carcinomas associated with multiple internal organ malignancies and metastasis. These are in contrast to those of Muir *et al.* (1967), Bakker & Tjon (1971) and the present case, where multiple malignant tumours did not metastasize.

Multiple cancers should be regarded as a special susceptibility of certain persons to carcinoma multiplex and not simply due to chance. This case is reported to stimulate follow-up of keratoacanthomas. Better understanding of the relationship between the skin pathology and internal malignancies may result. Careful observations and thorough clinical and post mortem studies of these patients may contribute to the knowledge of the malignant process.

ACKNOWLEDGMENTS

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Photography, courtesy of Medical Illustration Service, Veterans Administrative Hospital, Hampton.

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Senear-Usher syndrome with parathyroid adenoma

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SUMMARY

A patient with pemphigus erythematosus and parathyroid adenoma is discussed. He improved dramatically after excision of the tumour.

There has been recent interest in the association between bullous erythema multiforme (Basler, 1973), bullous pemphigoid (Parsons & Savin, 1968), pemphigus vulgaris (Saikia, 1972) and Senear–Usher syndrome (Saikia & MacConnell, 1972) with various neoplasms. A 46-year-old male patient presented with pemphigus erythematosus which was refractory to various forms of therapy including large doses of prednisone and parenteral methotrexate. In the course of the investigations, a parathyroid adenoma was discovered. After surgical removal of this tumour, the patient's skin disease dramatically improved, suggesting an association between the two entities. Such an association of these two uncommon entities has not been previously reported.

CASE REPORT

A 46-year-old Spanish-speaking Mexican–American migrant worker presented with a 2-year history of vesicles and bullae over the chest, back, upper arms, and face. The disease had been controlled with low doses of dexamethasone in the range of 5–10 mg/day by his private physician in Mexico. However, the condition had become more severe over the previous 2 months. No other pertinent history could be obtained.

Physical examination revealed a normally developed Mexican–American male with a bullous eruption over the chest (Fig. 1), back, and face. Tense, 0·5–2·0 cm bullae on an erythematous base were noted, with confluence of the lesions over the forehead and cheeks. A few lesions were also found on the scalp, but none was present in the mouth. Bilateral pterygia were present in the eyes. There were no palpable masses in the neck. Examination of chest, heart, abdomen and neurological system was unremarkable.

Skin biopsies stained with Haematoxylin and Eosin showed intraepithelial subcorneal clefts with acantholysis (Fig. 2). A perivascular, inflammatory infiltrate was also present within the upper dermis consistent with pemphigus erythematosus. Direct immunofluorescence staining demonstrated IgG in the intercellular spaces and at the dermo-epidermal junction. Laboratory screening tests showed a mild anaemia with a haemoglobin of 11·2 g/100 ml, increased calcium of 12·3 mg/100 ml, increased

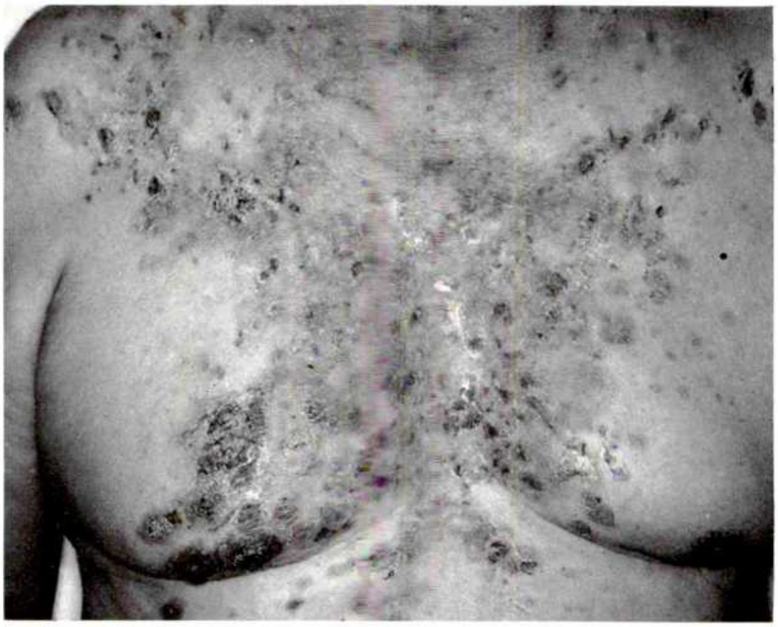

FIGURE 1. Vesicular and bullous eruption on erythematous base over chest.

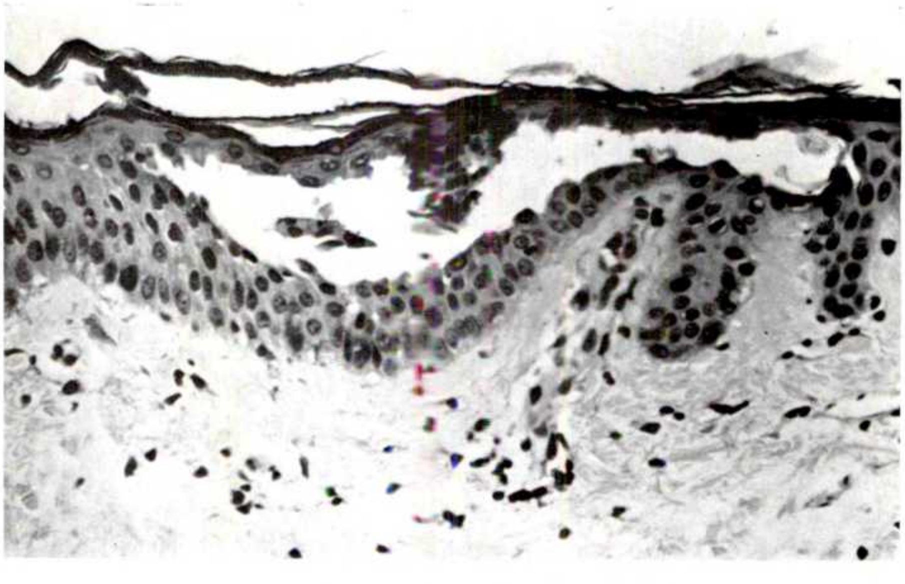

FIGURE 2. Acantholytic, intra-epithelial bulla (H & E, × 280).

alkaline phosphatase of 136 iu/ml, and a serum phosphorus at the lower limit of normal at 2·6 mg/100 ml.

The patient was placed on 160 mg of prednisone daily with initial improvement of old lesions, but appearance of a few new vesicles. An attempt to maintain this dosage during the investigation of his hypercalcaemia was unsuccessful; the pemphigus remained active and steroid side effects of melaena and transient psychosis appeared. The patient was started on 25 mg intramuscular methotrexate weekly and tapering of the steroid was begun.

Results of a parathyroid hormone assay showed a significantly increased value of 403 pg/ml, and the patient was transferred to the surgical service. Exploration of the neck revealed gross enlargement of the right inferior parathyroid, which was excised. The pathological report confirmed the diagnosis of a parathyroid adenoma.

The patient improved dramatically in the first 48 h following the operation, after nearly 6 weeks of unsuccessful therapy with conventional medications. He was discharged on the fifth post-operative day on 20 mg of prednisone daily with no active disease. The dosage of prednisone has gradually discontinued without new lesions appearing. New vesicles appeared approximately 3 months after the operation, however, and the patient was again placed on prednisone. The eruption has been under good control for the last 5 months on 25 mg every other day. There is no clinical or laboratory evidence of renewed parathyroid hyperactivity associated with the return of the skin disorder.

DISCUSSION

Three patients with Senear–Usher syndrome and malignant disease were recently reported by Saikia (1972). Although immunological investigations were not done, the clinical history of these patients suggested an interrelationship between their tumours and pemphigus antibodies (Saikia, 1973). Such an interrelationship had been demonstrated earlier with a malignant lymphoid neoplasm (Saikia, 1972). The fact that our patient's bullous disease responded dramatically to removal of the parathyroid adenoma after a prolonged and unsuccessful course of conventional treatment, suggests that antigens produced by the tumour may have aggravated the clinical condition by stimulating increased production of anti-epithelial antibodies. Though the pemphigus erythematosus did return, the course has been characteristically benign, and the process is under control with a low-dose, every-other-day steroid regimen.

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History of Dermatology

Sir Jonathan Hutchinson on toxic epidermal necrolysis—an historical note

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Accepted for publication 30 January 1974

> 'When anything turns up which is anomalous or peculiar, anything upon which the textbooks are silent and the systems and cyclopedias are dumb, I tell my students to turn to the volumes of Mr Hutchinson's *Archives of Surgery* as, if it is not mentioned in them, it surely is something very much out of the common.'
>
> OSLER (1900)

Toxic epidermal necrolysis is a generalized tender erythema which rapidly develops into an extensive exfoliation of the upper layers of the epidermis, giving the appearance of scalded skin. It is most commonly seen in infants and children. Lyell in 1956 analysed a group of cases, drew our attention to the important features, and gave the condition its present name. It has sometimes been claimed that the same condition had been earlier reported by the Ritter von Rittershain who in 1878 described 300 cases of exfoliative dermatitis in nurslings which he saw over a 10-year period in a foundling asylum in Prague. The condition started as a reddened exfoliating patch around the mouth, chin or lower face and then rapidly became a generalized erythema with shedding of large sheets of the upper epidermis. In areas where the epidermis did not come off, a superficial wrinkling was present. Fissuring and crusting were noted about the mucosa of the mouth, eyes and nose. Occasionally bullae were present. There was neither fever nor other constitutional symptoms. Suppurative sequelae were described by von Rittershain.

Toxic epidermal necrolysis is dramatic and is far from rare, and it would be surprising if other accounts than von Rittershain's had not been published. Jonathan Hutchinson appears to have described the same condition in the *Archives of Surgery* (1899) in a report entitled 'Fatal Dermatitis in an Infant attended by Bullae and general Epidermatolysis'. Although Hutchinson did not see the infant, he was sufficiently interested to ask for a complete report from Dr Bawden who testified at the inquest held to determine the cause of death. Dr Bawden supplied full particulars and a synopsis is provided by Hutchinson (Fig. 1). Hutchinson's comments on the differential diagnosis are as follows:

'Several diagnoses might be suggested. 1. Acute infantile pemphigus. 2. Erysipelas spreading from the excoriation on neck. 3. A dermatitis caused by the bromide. The absence of high temperatures and of congestion of skin seemed to distinguish the case from erysipelas. The prominent feature was loosening of the epidermis, so that it could be slipped about on the subjacent skin. The epidermis was loosened on the hands and feet, and 'might have been slipped off'. This epidermatolysis favoured the diagnosis of pemphigus. The vulva and nates were excoriated. The evidence of the child's father

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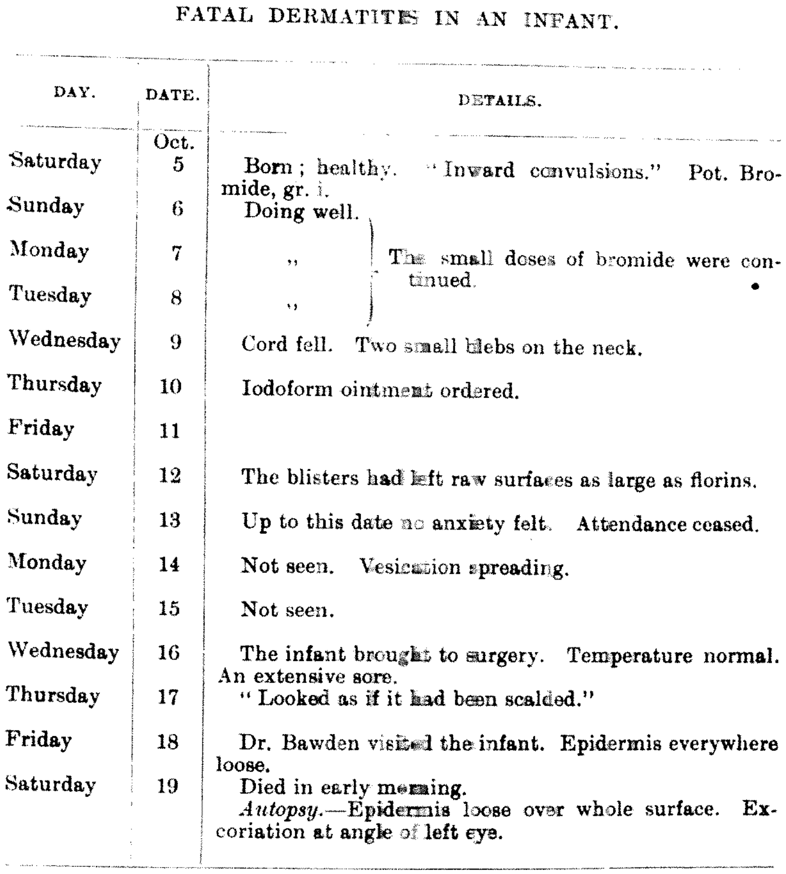


FATAL DERMATITIS IN AN INFANT.

DAY.	DATE.	DETAILS.
	Oct.	
Saturday	5	Born; healthy. "Inward convulsions." Pot. Bromide, gr. i.
Sunday	6	Doing well.
Monday	7	,, The small doses of bromide were continued.
Tuesday	8	,,
Wednesday	9	Cord fell. Two small blebs on the neck.
Thursday	10	Iodoform ointment ordered.
Friday	11	
Saturday	12	The blisters had left raw surfaces as large as florins.
Sunday	13	Up to this date no anxiety felt. Attendance ceased.
Monday	14	Not seen. Vesication spreading.
Tuesday	15	Not seen.
Wednesday	16	The infant brought to surgery. Temperature normal. An extensive sore.
Thursday	17	"Looked as if it had been scalded."
Friday	18	Dr. Bawden visited the infant. Epidermis everywhere loose.
Saturday	19	Died in early morning. *Autopsy.*—Epidermis loose over whole surface. Excoriation at angle of left eye.

FIGURE 1. Synopsis of case of 'Fatal Dermatitis in an Infant attended by Bullae and general Epidermatolysis' reproduced from Hutchinson (1899).

at the inquest was that it looked as if it had been scalded, but Dr. Bawden assured me that there was little or no redness.'

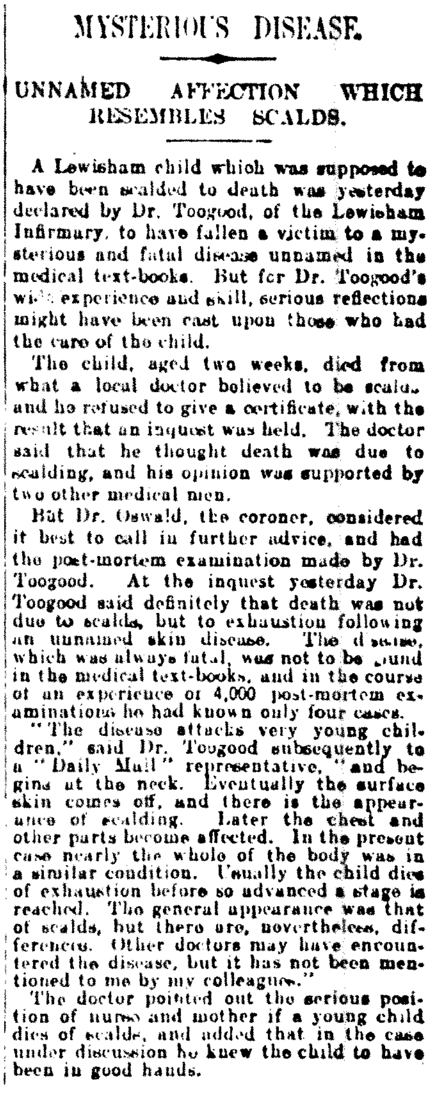
Hutchinson's personal and annotated *Archives of Surgery* are located in the Willan Room of the Royal College of Physicians in London. Affixed between pages 124 and 125 of Volume X is a letter from Dr Peter Cooper to Hutchinson, which reads as follows:

> Telegraphic Address
> Somebody London
>
> Jan. 6, 1905
> Stainton Lodge
> 35, Shooters Hill Road
> Blackheath 5-C
>
> Dear Mr. Hutchinson:
>
> Knowing what an authority you are on skin diseases and also all uncommon diseases too, I am sending you a cutting of a case that occurred with Dr. Bateman who called me in to see the case after death and from all appearances of the body I came to the conclusion that the child had fallen face down-ward in a bath of boiling water for the whole of the Epidermis of the face and body was either off or could be peeled off with the finger. The scalp alone appeared to be unaffected. The skin of the toes came off in my hand when I lifted up the foot to look at the buttocks.

Both Dr. Bateman and Mr. Bailey his partner were of the same opinion as myself, hence the Inquest. But if this is a rare skin disease as Dr. Toogood says, then it is a very interesting case and the sooner it is named and recorded the better, but I can hardly think Dr. Toogood can have had four cases of the same in 4000 post mortems as he says.

I should be very glad to have your valued opinion.

Yours sincerely,
Peter Cooper



MYSTERIOUS DISEASE.

UNNAMED AFFECTION WHICH RESEMBLES SCALDS.

A Lewisham child which was supposed to have been scalded to death was yesterday declared by Dr. Toogood, of the Lewisham Infirmary, to have fallen a victim to a mysterious and fatal disease unnamed in the medical text-books. But for Dr. Toogood's wide experience and skill, serious reflections might have been cast upon those who had the care of the child.

The child, aged two weeks, died from what a local doctor believed to be scalds and he refused to give a certificate, with the result that an inquest was held. The doctor said that he thought death was due to scalding, and his opinion was supported by two other medical men.

But Dr. Oswald, the coroner, considered it best to call in further advice, and had the post-mortem examination made by Dr. Toogood. At the inquest yesterday Dr. Toogood said definitely that death was not due to scalds, but to exhaustion following an unnamed skin disease. The disease, which was always fatal, was not to be found in the medical text-books, and in the course of an experience of 4,000 post-mortem examinations he had known only four cases.

"The disease attacks very young children," said Dr. Toogood subsequently to a "*Daily Mail*" representative, "and begins at the neck. Eventually the surface skin comes off, and there is the appearance of scalding. Later the chest and other parts become affected. In the present case nearly the whole of the body was in a similar condition. Usually the child dies of exhaustion before so advanced a stage is reached. The general appearance was that of scalds, but there are, nevertheless, differences. Other doctors may have encountered the disease, but it has not been mentioned to me by my colleagues."

The doctor pointed out the serious position of nurse and mother if a young child dies of scalds, and added that in the case under discussion he knew the child to have been in good hands.

FIGURE 2. Reproduction of newspaper cutting from the *Daily Mail* (1904).

A copy of the cutting referred to by Dr Cooper is reproduced (Fig. 2) (*Daily Mail*, 1904).

The reference to both cases having an inquest to determine the cause of death, as scalding was suspected, reminds one of Walker's report (1959); in one of her cases the house physician refused to sign a death certificate because he could not convince himself that death was not from some unnatural cause—'for example, the ministrations of a witch doctor'.

The reports of both von Rittershain and Hutchinson describe a generalized erythema with extensive superficial peeling in infants and children, giving an appearance of scalding. Fever was not present. Occasionally blisters were seen. All things considered, it seems most likely that Lyell's syndrome, Hutchinson's 'Fatal Dermatitis in an Infant attended by Bullae and general Epidermatolysis', and von Rittershain's 'Exfoliative Dermatitis in Nurslings' are all examples of the reaction pattern in the skin known as toxic epidermal necrolysis.

ACKNOWLEDGMENTS

The author thanks the curators of the Willan Room and the Trustees of the British Museum for providing material for this paper.

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British Journal of Dermatology (1974) **91**, 473.

Comment

MYCOBACTERIAL SKIN ULCERS

In some areas of the tropics chronic skin ulcers caused by *Mycobacterium ulcerans* are a common and serious disorder. Since their first description, in Australia in 1948 (MacCallum *et al.*), there have been numerous investigations into the method of transmission of the organism, the pathogenesis of the lesions and the role of chemotherapy in treatment.

Both sexes and all age-groups are affected, and most patients present with a single cutaneous ulcer which is painless, and which has a characteristic undermined edge (Uganda Buruli Group, 1972). Lesions are usually on the arms or legs but may occur on any area of the body except the palms and soles. Distinction between these ulcers and tropical ulcers is seldom difficult, since tropical ulcers are painful, do not have undermined edges, and are rarely seen above the knee.

Sometimes lesions are seen at the earlier pre-ulcerative stage, when they are small, painless, subcutaneous swellings, attached to the skin but not to the deep fascia. Such lesions may closely resemble those seen in other diseases, for example subcutaneous phycomycosis, but clinical diagnosis is nevertheless possible in most cases, and may be confirmed by biopsy.

Microscopy of pre-ulcerative lesions shows clumps of organisms surrounded by areas of bland necrosis of the subcutaneous tissues. As the lesions progress, the necrosis extends slowly through the subcutaneous tissues, over a period of many weeks, while the blood supply to the overlying skin becomes cut off with resulting ulceration and secondary infection. Some lesions extend widely and may involve an entire limb or a large area of the trunk. Such extensive loss of skin and subcutaneous tissue can lead to the death of a young child, but usually slow healing occurs to leave gross deformities and contractures. Although there may be considerable local destruction of tissue, penetration of the disease through the deep fascia is uncommon; regional lymph nodes do not become involved, and systemic spread is very rare.

Myco. ulcerans is sensitive to a number of drugs *in vitro*, but the clinical results of chemotherapy have been disappointing; and the disease remains one of few infections for which the definitive treatment is surgical. Pre-ulcerative lesions may be excised under local anaesthesia, in dispensaries or by the roadside. Ulcerated lesions require hospitalization so that diseased tissue can be excised and the denuded areas covered by skin grafts. Within two endemic areas in Uganda regular clinics for the disease have been set up in the villages. This encourages diagnosis and treatment at the early pre-ulcerative stage, and has obvious advantages both in the prevention of the crippling deformities which may follow advanced disease and in reducing the need for extensive surgery and prolonged hospitalization.

Two controlled trials have been undertaken in Uganda to determine whether BCG vaccination confers protection against *Myco. ulcerans* infection. In the first, carried out within Kinyara refugee settlement, the overall protection rate was found to be 50% during the first year; but within different sectors of the settlement the protection rate varied inversely with the incidence of the disease. A second trial, on a larger population, is not yet complete but preliminary results confirm a 50% protection rate.

Myco. ulcerans is an unusual pathogen in that it grows preferentially at 32–33°C and on primary isolation is usually inhibited at 37°C. This preference for temperatures lower than body temperature may explain the restriction of lesions to the subcutaneous tissues. Evidence from family studies and case-clustering analyses strongly suggests that the organism is usually not transmitted from one person to another. However, *Myco. ulcerans* has never been isolated from any natural source other than diseased human tissue, and the non-human source of infection remains unknown. It is the need to identify this source which has stimulated recent intensive epidemiological investigations.

Perhaps the most tantalizing clue is the geographical association of the disease with swamps and watercourses. For example, in Uganda (where 600 cases were reported during 1970) the disease is confined to swampy areas close to the Nile; and in New Guinea most cases have come from a group of villages near the Kumusi river. Another epidemiological characteristic of the disease is its tendency to occur in outbreaks, such as was recently seen at Ibadan, Nigeria, where a localized outbreak followed the damming of a stream. In Kinyara refugee settlement, Uganda, nearly 300 out of 2500 refugees developed the disease during a 3½-year period.

The disease has a higher incidence in children than in adults, the maximum frequency being in the 5–15-year-old age-group. The striking feature of the age–sex distribution in Uganda is that, whereas among children the incidence in the two sexes is similar, among adults the incidence in females is more than twice that in males. Also, in Uganda, there is a marked seasonal pattern which differs in males and females. It is difficult to reconcile the sex differences in incidence and seasonal distribution with a source of *Myco. ulcerans* in the vicinity of a family's dwelling. It seems more likely that transmission of the disease occurs away from the houses in relation to some domestic or agricultural activity in which males and females have different roles.

There have been numerous hypotheses to explain the method of transmission of the disease, and mosquitoes, birds, reptiles, and rodents have all been invoked as possible agents. However, in the light of available data it seems reasonable to conclude that transmission depends on proximity to swamps. One hypothesis which has recently been investigated is that *Myco. ulcerans* occurs in soil and on vegetation in and around the swamps, and that people come into contact with the organism through activities such as collection of domestic water and washing of clothes (Barker, 1973). Many hundreds of vegetation and soil samples have been collected, and large numbers of mycobacteria isolated from them, but *Myco. ulcerans* has not been found.

An eventual solution to this fascinating problem must surely come from perseverance in bacteriological and epidemiological studies. But it remains to be seen whether the solution will depend on improved techniques for isolation of *Myco. ulcerans*, or on the formulation of new hypotheses.

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Obituary

M.RUITER

Professor M.Ruiter of Groningen died on the 2 June 1974. He had been an honorary foreign member of the British Association of Dermatology since 1966.

Society Proceedings

Skin Microbiology Club

At a meeting of the Skin Microbiology Club, held at St John's Hospital, Leicester Square, London on 25 April 1974, Dr A. Knight (St John's Hospital) described his work on Experimental Human Fungous Infection. Dr Knight, who started the work in California under Dr R. B. Stoughton, discussed the production of lesions on the human upper arm by *Trichophyton* species and the value of such lesions in studying prophylactic treatment. In an attempt to refine the investigations, a change in technique was made by growing the fungi on human stratum corneum adhering to sticky-tape. Persistence of an antimicrobial compound on human skin was studied by sampling the treated skin of the respondent at specified time intervals.

The discussion was begun by Dr R. Marples (Central Public Health Laboratory) who reported briefly on his experimental studies in Philadelphia and suggested that the initial reaction, seen in experimental lesions, was an allergic rather than an invasive one. The use of a corticosteroid could convert the reaction to an invasive state. The subsequent discussion centred on the nature of the skin reaction and the difference in response expected from drugs in relation to prophylactic or to curative treatment.

Correspondence

LICHENOID DRUG REACTION FROM METHYLDOPA

Sir, Lichenoid drug eruptions with the histological features of lichen planus have been attributed to methyldopa although no provocation tests were done (Almeyda J. & Levantine A., 1971). Church (1973) describes a seborrhoeic dermatitis-like eruption from methyldopa which had been confirmed by further exposures to the drug, but without any histopathological studies. We wish to report tongue ulceration with features of lichen planus in three patients and a fixed drug eruption in one patient which we consider were caused by methyldopa.

Case 1. Female, aged 64 years. Methyldopa had been taken for over one year before tongue ulceration developed in March, 1971. Psoriasiform scaling of the scalp and dry eczema of the arms appeared about the same time. She was treated with oral prednisolone but the tongue did not improve until the methyldopa was stopped in September, 1971 and took 5 months after that to heal completely. The eczema-like rashes cleared more rapidly. Two biopsies from the tongue taken during 1971–1972 revealed chronic inflammation only.

Case 2. Female, aged 66 years. Methyldopa had been taken for several years before the development of tongue ulceration and a seborrhoeic dermatitis-like eruption of the scalp in September, 1972. A biopsy of the tongue in 1973 was reported as consistent with erosive lichen planus. Treatment was commenced with oral prednisolone without any real improvement. Not until the methyldopa was stopped did the ulceration of the tongue start to improve immediately but complete healing did not occur for 4 months.

Case 3. Female, aged 68 years. Methyldopa had been taken for several years until mouth ulceration developed in October, 1970. A biopsy at that time was reported as consistent with the appearance of lichen planus. Eczematous rashes of the dorsa of feet, chin, forearms and upper back were present and disappeared quickly when the methyldopa was ceased in August, 1973, although the tongue did not completely heal for 4 months after that.

Case 4. Female, aged 57 years. Methyldopa had been taken regularly for over 2 years when in October 1973 a violaceous, erythematous, oedematous fixed drug eruption 15 × 13 cm appeared on the inside of the upper left groin. Similar but smaller patches on the inside of the right forearm and upper left chest had appeared several weeks after the initial patch. Amitriptyline, quinine and indomethacin were being taken and these drugs were stopped, but the methyldopa was continued. Three weeks later there was no improvement and the methyldopa was then stopped with immediate relief from burning, itching symptoms. The patches on the arm and chest cleared completely in 4 months, but 6 months after methyldopa cessation, an asymptomatic, pigmented, thickened patch remained within the groin.

A biopsy of the initial lesion at its inception revealed hyperkeratosis, a prominent granular cell layer, acanthosis, saw-toothed rete ridges and a chronic inflammatory infiltrate of histiocytes and lymphocytes associated with melanin granules within an oedematous dermis. These appearances were considered consistent with those of a lichenoid drug eruption.

Lichenoid drug eruptions and fixed drug eruptions are similar inflammatory events, since skin changes produced by them persist over long periods (Bruinsma, 1973) and since they resemble each other closely in their histopathological appearances (Montgomery, 1967). We would emphasize the diagnostic difficulties in view of the long time required for healing of the tongue ulceration after withdrawal of the methyldopa in three of our patients.

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DIGITAL VASCULAR DISEASE AND MALIGNANT DISEASE

SIR, I have recently seen three patients with digital occlusive vascular disease in association with malignant disease and suggest that it might be more than a chance relationship.

Case 1 was a 62-year-old woman who had a reticulum cell sarcoma. Early on in the course of her illness she developed pain and paraesthesiae in several fingers. Her right index and middle fingers had blotchy cyanosis of the tips and a purplish line of demarcation between ischaemic and normal skin. There were splinter haemorrhages beneath all nail plates. Reactive hyperaemia was absent distally. Digital signs persisted until her death several months after symptoms first appeared.

Case 2 was a 74-year-old woman who developed numbness and tingling in all her fingers. These showed cyanosis, absent reactive hyperaemia of the tips and numerous subungual splinter haemorrhages. The signs varied in degree for many months. Ultimately she was found to have extensive intra-abdominal carcinoma from which she died.

Case 3. A 66-year-old woman who had a gastric carcinoma, noticed pain and paraesthesiae in the fingers of her left hand. The finger tips were cold, blue and tender. Splinter haemorrhages were seen in the nail beds. Reactive hyperaemia was absent. Symptoms lessened after the tumour had been removed.

None of these patients gave a previous history of Raynaud's phenomenon or treatment with vasoconstrictor drugs. The sudden onset of symptoms in all cases was unrelated to cold exposure. There was no evidence of any of the conditions predisposing to digital ischaemia (Birstingl, 1971). Extensive studies were undertaken to exclude dysproteinaemia, collagen disorder, endocarditis, and proximal arterial disease. The changes suggested a digital vasculitis, but unfortunately the digital vessels were not examined histologically, or by immunofluorescence studies. The ischaemia in these three patients was less severe than in those previously reported to be associated with malignant disease by Hawley, Johnston & Rankin (1967), in whose patients it usually progressed to frank gangrene.

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M.3 Unit
H.M.Palmer

ACKNOWLEDGMENTS

My thanks are due to Dr S.Oleesky and Dr. A.J.Ralston for allowing me to present details of patients under their care.

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Book Reviews

Cryosurgery of Tumors of the Skin and Oral Cavity. Setrag A. Zacharian (1973) Springfield, Illinois: Charles C. Thomas. Pp. xvi × 293, illustrated. Price £10.90.

British dermatologists reading this book are likely to ask themselves several questions. Are we, for instance, neglecting an important means of treating our patients by not using the tools of cryosurgery? The answer seems to be 'yes' only if one wishes to treat malignant tumours by this technique. Although the apparatus described by the author for applying liquid nitrogen is more convenient and possibly safer to use than cottonwool on a stick, the most important innovation of recent years has been the use of hypodermic thermocouples to measure accurately the degree of freezing below and around the lesion being treated. This allows the treatment of a predictable margin of normal skin similar to that obtained by ionizing radiation. If the cure-rate of cryosurgery compares with that of the established methods of treating tumours, it has advantages of simplicity, safety and economy which make it very attractive. It seems likely that the author is promoting a cause which will succeed.

The question which kept occurring to me as I read the book was why did the publishers not employ a firmer editor to revise the vocabulary and clarify the style of writing. One can excuse the cryojargon, which is not excessive, and the occasional spelling errors, but a sentence like 'A number of parameters, collectively, induce cellular damage in a freezing experience' (page 30) is not only turgid, but also distorts the proper meaning of 'parameter' and of 'experience'. One might also quibble at the use of pathogenesis in 'The Pathogenesis of Skin Cancer following Cryosurgery' (chapter 4) and of variance in 'The clinical variance of this disorder may vary' (page 98). Writing a book, as the author says, is not an easy task and enthusiasm for the subject is not sufficient to overcome all the difficulties.

The first section deals with the effects of low temperatures on cells, tissues and skin cancer, with a useful selection of references. It contains detailed accounts of experiments which would have been better published in a journal and only summarized here. One must applaud the author for investigating experimentally the pathological basis of the treatment, even if a certain lack of professionalism shows through. Figure 2-12 purports to show basal cell carcinoma actively growing in tissue culture, but looks much more like fibroblast proliferation. The more important sections contain practical details and illustrative case histories of the treatment of benign and malignant tumours of the skin, haemangiomas, and of cryosurgery in the nasal, oral and pharyngeal cavities. The author has co-opted three surgeons to write on their special areas, but has not used his editorial powers to avoid overlap between the different writers. The rather elementary description of the clinical features of the different conditions treated suggests that the book has been written for surgeons, or general practitioners, rather than for dermatologists.

The book is elegantly produced but the quality of the illustrations is, in general, very poor. Those dermatologists particularly interested in the treatment of skin tumours will wish to read it, but one suspects that the majority will go on using liquid nitrogen by means of cottonwool on a stick.

K.V.SANDERSON

Lehrbuch der Haut und Geschlechtskrankheiten. F. AND E.FLECK (1973) 4th Edition. Berlin: VEB Verlag Volk und Gesundheit. Pp. 499, 231 illustrations. Price (2nd Edn £5.23.)

Venereology occupies only about thirty of this textbook's pages. The work has an historical introduction with a charming picture of a thirteenth century stone carving in Constance Cathedral showing possibly the earliest representation of a dermatologist with pot of ointment and magnifying glass. The book ends with an account of DDR law relating to dermatovenereology. The rest of the book is traditional in approach and sometimes excessively academic in arrangement. Many of the opinions are out of line with present thought and practice. For example, keratoacanthoma is described as a precancerosis although it is briefly stated that spontaneous involution is possible. Pemphigoid is classed as a variant of pemphigus without reference to evidence to the contrary, and there is no mention of gluten sensitivity in dermatitis herpetiformis. The numerous black and white illustrations are adequate although mostly small. Since this is the fourth edition of this book in 12 years there is clearly a demand for it in East Germany, but it has nothing special to offer us. It is too comprehensive for our undergraduates and is too didactic for the postgraduate.

P.J.HARE

Psychosomatic Medicine. A.MUNRO (Ed.) (1973) Edinburgh and London: Churchill Livingstone. Pp. 95. Price £1.75.

This slim and inexpensive volume brings together twelve articles originally published in the 'Practitioner'. Dr W.Susskind of Glasgow writes competently on Psychosomatic Aspects of Skin Disease; he rightly implies that in most conditions in which psychogenic factors can be implicated, these play merely a contributory, aggravating or perpetuating role, but does not stress that the demonstration that such a factor makes a condition such as chronic urticaria worse, and does not justify classifying this troublesome disorder as psychosomatic.

Professor Alistair Munro, the editor, contributes an interesting introduction and an article on 'Gynaecology'; Dr C.M.Harris writes on 'Tiredness and Headaches'; Dr. P.Harper on 'Weight Disorders'; and Dr P.Pinkerton on 'Asthma'. All these authorities are concerned with problems encountered in dermatology clinics many times each day in patients presenting with skin lesions, to which they may or may not be causally related. This convenient and easily digested book will provide those many dermatologists who are interested in patients rather than cases, with practical help and with food for speculation.

ARTHUR ROOK

Consultations in Dermatology II. WALTER B.SHELLEY (1974) Philadelphia London, Toronto: Saunders. Pp. x+333, illustrated. Price £10.65.

Dr Shelley's first volume of 'Consultations', published in 1972, has deservedly enjoyed wide popularity. The earliest example of the use of a collection of case reports, each lucidly and learnedly discussed, as a basis for what amounts to an informal textbook of dermatology, was by Clément Simon, of the Hópital St Lazare, Paris. Simon published in 1930 a volume the title of which may be translated as 'Letters to a General Practitioner on Dermatology and Venereology'. This book was so successful that Dr Simon published a second volume of letters in 1935. In each of his letters Simon first summarized a case history, then reviewed some relevant literature, and finally assessed the diagnostic and therapeutic problems involved. His two books still make enjoyable and not unprofitable reading.

Walter Shelley's approach is similar in principle, but differs in detail. He takes the reader on a ward round, and illustrates each case summary with an excellent photograph by Edward F.Glifort. (Why do so few authors acknowledge their photographers by name?) He then provides a stimulating and exceptionally well informed discussion of the case, followed by an international bibliography, remarkable for its scope, and enhanced by brief comments on each paper. Some of the conditions covered in this second volume are relatively rare, and it is essentially a book for dermatologists; it is indeed also a book essential for all dermatologists, for one would be inclined to question the credibility of even the best informed, if he claimed he had learned nothing from it.

Inevitably some questions are left unanswered, but it would have been interesting to hear in what proportion of the author's own cases of chronic urticaria he establishes a cause, acceptable as such beyond any reasonable doubt. It would also be interesting to know how he tackles the recurrent keratoacanthoma.

This excellent book is strongly recommended as a means of keeping up to date with the minimum expenditure of intellectual effort.

ARTHUR ROOK

MEDIZINISCHE DIDAKTIK, Erläutert durch beispiele aus der Dermatologie. WOLFHARD SAUERBREY (1974) **Heidelberger Taschenbücher.** Berlin, Heidelberg, New York: Springer. Pp. 237. Price £2.85.

The 'Heidelberger Pocketbooks' enjoy popularity among German speaking medical students and practitioners because they provide at low cost a compact yet comprehensive account of most medical specialities. It is to the credit of the editors of this series that they have now published a treatise on medical didactics written by a young dermatologist from Munich. The author's former chief at Frankfurt, Professor Nasemann, himself a disciple of one of Germany's finest dermatological teachers, Alfred Marchionini, rightly stresses in the introduction to this book that most University teachers require training not only in scientific discipline but also in didactics, the science of teaching.

Students can easily acquire factual knowledge by reading and observation but most need guidance in the evaluation and critical interpretation of such data. Didactics in medicine are concerned not only with the utilization of modern teaching aids to impart information, but also with the application of 'formal logic' and the scientific method and problems of differential diagnosis and therapeutics. Academic training should equip students to tackle any problem in his chosen field by teaching him methods rather than ephemeral data.

This little book fills an important gap in the medical literature and general academic teaching outside the field of formal philosophy. There is much to be said for including in the pre-clinical curriculum, or the Sixth Form syllabus for intending science students, the philosophy of formal logic and to allow the senior teaching staff of all faculties access to the same privileged knowledge. The time such training would save on committee work alone would more than make up for the time spent acquiring it, not to mention the intellectual satisfaction to the beholder.

Dr Sauerbrey is not the first to criticize the lack of any formal training for University teachers who are expected to lecture, to lead discussion groups, to plan teaching courses and programmes, to evaluate the research of others and to conduct their own, i.e. to be allround dilletantes; but he must be one of the first to advance the case for introducing medical didactics as a formal discipline using his own speciality as an example for its practical application. He evaluates critically most modern teaching aids, such as teaching machines, tape and slide programmes, films and videotapes, and includes their costing.

This book is the thoughtful effort of an original, inquiring mind. It may lack the cold mathematical clarity and definition of a Spinoza, it occasionally misses the obvious or weakens a strong plea by overstating it; but it should be obligatory reading for those responsible for organizing undergraduate and postgraduate teaching, in fact for all who teach medicine and its basic sciences.

The author's style is not easy to read, appears often telescoped and unnecessarily fragmented. Possibly limitation of space and preoccupation with trivia have occasionally led to the ommission of clearly defined principles. These are minor criticisms of an important and long overdue book on a very topical subject.

G.HOLTI

Thesis

Irritant Cutaneous Effects of 'Gentian Violet'. A Clinical and Experimental Study.

Thesis presented by Håkan Mobacken, Göteborg University, Sweden, 1974.

Gentian violet, a triphenylmethane dye, is a mixture of hexa- and penta-methylpararosaniline chlorides. It is widely used and is considered to be safe. The author of this thesis noticed that in some patients it gave rise to irritation and even to necrotic ulcers. Experimental studies in two such patients confirmed that in inflamed skin 1% aqueous solution of gentian violet could indeed induce a necrotic reaction. It was then shown that similar reactions could be produced in 'stripped' but otherwise healthy skin in man and the guinea-pig, but not in healthy 'unstripped' skin.

Further experiments established that triphenylmethane dyes are capable of causing tissue damage in concentrations well below those in which they are commonly prescribed.

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Review

Bacterial infections in diabetes mellitus

A glance through the papers on this subject is a treat not to be missed by those who like circular arguments and contradictions. There seems always to have been confusion—for a nice review of the muddle as seen in 1935, read Pillsbury & Kulchar. The present review can only point to the main tangles but cannot unravel them. The line of reasoning should be simple; first to show that diabetics are more prone to infections than others' and then to ask why this should be. In fact, the clinical basis for the first part, with some exceptions, is not solid, and most of the work has gone into looking for reasons, mainly immunological, why diabetics might be expected to be liable to infection, rather than showing that they actually are. A grasp of this general background is needed to see the link between skin infections and diabetes in its proper setting.

Flynn (1935) pointed out that deaths, both from sepsis and from pulmonary tuberculosis in diabetics fell when the insulin era began in 1922. He credited this to insulin itself. The same trend continued and is beautifully shown in the paper by Entmacher, Root & Marks (1964). By 1966, Malins could find no excess of deaths caused by infections among diabetics; although, applying the same technique to the period before streptomycin, he had found a raised death rate from pulmonary tuberculosis in female diabetics. Pell & D'Alonzo (1970) followed a large group of diabetics and controls over a period of 10 years; infection was not an important cause of death in either group. Infections were not even mentioned in a discussion of the complications affecting patients who had regularly attended the same diabetic clinic for 40 years (Oakley *et al.*, 1974). Despite this, in diabetic coma, infection is still important both as a trigger and as a cause of death (Biegelman, 1971; Biegelman & Warner, 1973). As control of diabetes has got better, so the danger of major infections has faded, although the risk is still there for patients in coma.

As H.G.Wells has said (Koestler, 1964) 'the forceps of our minds are clumsy things and crush the truth a little in taking hold of it'. But can a different handling of the same facts explain why the experts still contradict each other about whether diabetics are really more prone to pyococcal infections than normal people, or whether those that they do have are more severe? Most of the references on this point are no more than quotations of opinions set out in textbooks. However, Williams (1942) tried to answer these questions and held that skin infections occurred no more often in diabetics than non-diabetics. On the other hand, Farrer & Macleod (1960), in a huge survey of staphylococcal infections acquired in hospital, found far the highest rate in patients with diabetes mellitus. Diabetes was also noted particularly often among patients admitted with staphylococcal infections. During the Bedford study (Keen, 1966), the survey population were asked about recent skin sepsis; answers were correlated with blood sugar levels estimated 2 h after a glucose load. Rather more (about 9% of 104 patients) of those with levels above 200 mg/100 ml admitted to skin sepsis than did those (about 5% of 1137 patients) with levels of less than 120 mg/100 ml. Against this, in another large survey (Welborn *et al.*, 1966), a population of nearly 4000 was screened for diabetes; a standard inquiry for recent skin infections showed no difference between non-diabetics and diabetics, both already known and newly found. Infections were not mentioned accompanying polydipsia and polyuria as a symptom of patients before their diabetes was found (Wilkerson & Krall, 1947).

It all seemed simple when Smith, O'Connor & Willis (1966) proved that diabetics, especially those on insulin, carried staphylococci in their noses more often than controls. This would explain why they had more sepsis but, annoyingly, Somerville & Lancaster-Smith (1973) did not confirm this finding. Again there is the struggle to find theoretical reasons for attractive ideas which are not fully supported clinically.

The special liability of diabetics to urinary tract infections was obvious when Robbins & Tucker (1944) reported that 307 diabetics had a far higher prevalence of renal infections at autopsy than 2800 non-diabetics. This was borne out by the even larger series of Edmondson, Martin & Evans (1947). But the diabetes itself may not have been the cause. Diabetics were then regularly catheterized, and this, with the poor emptying of a neurogenic bladder, would make it hard to escape a urinary tract infection. Later studies (Huvos & Rocha, 1959; O'Sullivan *et al.*, 1961), looking at the frequency of bacteriuria, have found no difference between diabetics and controls. Animal work adds its usual sharp lesson. Browder & Petersdorf (1964), with intravenous *E. coli* injections, found that alloxan diabetes made it easy to give rats pyelo-nephritis: this was not due to high blood sugar levels but to the damage to the kidneys from the alloxan itself. Indeed Krizek & Davis (1964) injecting *E. coli* subcutaneously into rats showed that these achieved higher blood levels in non-diabetic than in diabetic animals. Pseudomonas infections of alloxan diabetic mice were no more severe than in controls (White & Noble, 1974). Diabetes is still in the list of conditions predisposing to gram-negative rod septicaemia (Lewis, 1972). This is odd in view of the poor gram-negative growth at high sugar levels (Robson & Heggers, 1969), and may be a sequel of the once high rate of urinary infections. However diabetics do seem prone to the less common infections such as *E. coli* pneumonia (Tillotson & Lerner, 1967) and *E. coli* cholangitis (McFadzean & Yeung, 1968).

Mild erythrasma is common in most populations (Somerville *et al.*, 1970) but Montes *et al.* (1969) noted diabetes in nine out of nineteen unusually extensive cases of erythrasma; in some it was the presenting feature of the diabetes. Somerville & Lancaster-Smith (1973) have confirmed that erythrasma is especially common in diabetes but found no extensive infections. They also found that the carriage of fluorescent diphtheroids on clinically normal skin was more common in diabetics than in the normal population. These organisms can ferment glucose, but other factors, particularly obesity, may be important.

The role of diabetes in other infections is less certain. In one series of patients with leprosy (Finlay-Jones & McComish, 1972) an unusually high prevalence of diabetes was found, but this was probably a reflection of the high prevalence of diabetes in Australian aborigines. *Aeromonas hydrophila* infection has been reported in a diabetic (Levin, 1973). Perianal skin gangrene is a rare and severe complication of amoebic dysentery; it has been seen in a diabetic (Lord, 1973). Meliodosis seems to favour diabetics (Jackson, Moore & Sanford, 1972); possibly protothecosis does too (Davies, 1972).

Lassar (1904) naively felt that a high blood sugar level might help staphylococci to grow. This was soon dismissed by Handemann (1911) who found that adding glucose to blood did not favour the growth of pathogens. The picture became confused again when Cruickshank & Payne (1949) showed that *pneumococci* grew more quickly in the blood of rabbits with alloxan diabetes than in the blood of normal rabbits. Curiously, the organisms grew better in the non-diabetic rabbits' plasma, hinting at a failure of diabetic white cells to check the organisms. Things are still more complicated now. Robson & Heggers (1969), using plasma with varying amounts of glucose added, noted that gram-positive organisms thrived on high sugar levels while gram-negative rods did not. Above a plasma glucose concentration of 400 mg%, gram-negatives did not grow well enough to reach what they felt was the critical level to cause clinical infection.

The relationship works both ways. Not only do high sugar levels inhibit gram-negatives, but gram-negatives contribute to low sugar levels by depressing gluconeogenesis (Lanoue, Mason & Daniels, 1968). Robson (1970) found that if the blood sugar of an infected diabetic patient was low, the organism was likely to be gram-negative. This was reliable enough to choose an antibiotic, bearing in mind the way that oxytetracycline can itself lower the blood sugar by prolonging and intensifying the action of insulin (Hiatt & Bonoris, 1970).

Much effort has gone into studies of polymorph function. Most workers, with some exceptions (Crosby & Allison, 1966; Miller, Baker & Kaye, 1968), find something wrong with the diabetic polymorph; but is it the blood sugar, the presence of ketones, or the blood pH which is responsible? Bagdade, Root & Bulger (1974) may have solved this by looking at diabetics who were poorly controlled but not ketotic. Leukocyte function tests on cells from these patients showed both depressed phagocytosis and a decreased rate of killing a test organism. Both tests improved when the diabetes was treated, but the microbicidal rate stayed rather less than the control values. A factor was found in the serum of these patients which could suppress polymorphs from controls; it may have been glucose, as the same effect followed adding glucose to the system provided that this was more than 250 mg/100 ml. This fits very nicely with earlier work on the non-ketotic rats with alloxan diabetes (Drachman, Root & Wood, 1966) whose susceptibility to infection and depressed phagocytosis was also due to the high glucose levels. Mowat & Baum (1971), finding deficient chemotaxis in polymorphs from diabetics, had shown that this came back to normal after incubation with insulin and glucose together. This abnormality did not relate to the presence of keto-acidosis.

These changes are not found only in the test-tube. The early granulocyte phase of the local cellular response is delayed in poorly controlled diabetics (Perillie, Nolan & Finch, 1962) and leukocyte migration is reduced (Kontras & Bodenbender, 1968; Brayton, Stokes & Schwartz, 1970). The leukocytes of rabbits with hyperglycaemia stick poorly to the vascular endothelium and migrate less in response to experimental skin damage (Ainsworth & Allison, 1970). The thickened capillary basement membrane of the human diabetic (Siperstein, Unger & Madison, 1968), may slow down leukocyte movement and block the diffusion of insulin and glucose to white cells already outside the vessels. Slow healing (Abbey, Cohen & Shklav, 1972), reduced collagen formation (Glickman, Smulow & Moreau, 1967) and malfunctioning of mast cells (Sheldon & Baver, 1960) may also be found in diabetics.

Chemotaxis and phagocytosis are dependent on energy from glucose metabolism which is depressed in the diabetic polymorph (Martin *et al.*, 1953). This is easy to understand and sounds possible, but there are those who feel that the ketosis itself is important (Perillie *et al.*, 1961, 1962; Bybee & Rogers, 1964). However, pH changes do not seem to be enough to account for the problems of the diabetic polymorph (Bryant, Desprez & Vanway, 1966).

The finding (Brody & Merlie, 1970) that the lymphocytes of diabetics have a depressed glucose metabolism and respond poorly to phytohaemagglutinin, has not been confirmed (Ragab, Hazlett & Cowan, 1972). Diabetes and pregnancy both predispose to candidiasis but there is little evidence that diabetics have a depressed cellular immunity of the sort now being shown to exist in pregnancy (Finn *et al.*, 1972, Purtilo, Hallgren & Yunis, 1972).

Confusion also reigns on the subject of antibody responses. Richardson (1933) said that diabetics were less able than non-diabetics to form agglutinin after inoculation with typhoid vaccine. In mice anyway, (Dolkart, Halpern & Perlman, 1971), there was no difference in antibody response to bovine albumen between alloxan-diabetic animals, both untreated and on insulin, and normal mice. In fact, human diabetics, on oral hypoglycaemics or on a diet, seem to have higher IgA levels than either insulin treated diabetics or non-diabetics (Farid & Anderson, 1973). IgG levels, though within normal limits, seem to relate to the daily dose of insulin. The amount of immunoglobulin on the surface of leukocytes from diabetics is normal (Tornblom & Wilkund, 1967; Mowat & Baum, 1971).

Is the diabetic skin likely to help invaders? Adult diabetics are both heavier and fatter than controls (Butterfield, 1966), making them liable to intertrigo with its flora of *staphylococci* and *streptococci* (Smith & Waterworth, 1962). In diabetics, 'toes are a greater hazard than teeth' (Joslin, 1941).

Bacterial invaders through the splits caused by tinea pedis are a clinical problem, the micro-angiopathy of diabetes probably helping infection in the way that adrenaline induced ischaemia does (Evans, Miles & Niven, 1948). The flora of peripheral vascular ulcers was described by Friedman & Gladstone (1969).

Skin pH changes, even if these were to follow blood values, do not affect the skin flora much (Noble, 1968). If the effect of blood sugar levels on infection is hard to interpret, then that of skin sugar levels is far harder. Skin samples containing both dermis and epidermis have sugar levels directly related to the blood levels (Peterka & Fusaro, 1966). The ratio of skin to blood glucose was slightly higher in diabetics (about 70%) than in normal people (55%), but they could not confirm earlier reports that diabetics with skin infections had particularly high ratios. Epidermal glucose also relates directly to blood levels (Shragger *et al.*, 1962). Palmar sweat even in diabetics, contains little, if any, glucose (Lobitz & Osterberg, 1947). There is no evidence about the effect on the skin flora of the curious heavy sweating of the face and chest seen in some diabetics (Watkins, 1973). In other words, there is information about the glucose content of most parts of the skin except the hair follicles in which the *staphylococci* must multiply before reaching the critical pus-forming dose (Elek & Conen, 1957).

There is only one proper study of the diabetic skin flora—that of Somerville & Lancaster-Smith (1973). Their ninety-eight adult diabetics were under good diabetic control, but there was no matched series of non-diabetics. *Staphylococcus aureus* was not commoner in the nose or on the skin than in healthy populations studied by other workers. However, *Sarcinae* and *micrococcus* group 7 were found more often, surprisingly since these do not ferment the glucose during growth. The dose of insulin needed, the duration of the disease, and the presence of medical complications made no significant difference to the skin flora.

In fact, apart from the higher incidence of fluorescent diphtheroids, their diabetics had a skin flora similar to non diabetics of the same age. They did not confirm the earlier report (Marples, 1965) of reduced aerobic diphtheroids, on the skin of diabetics. Using a replica method, it has been found that diabetics do not carry a higher cutaneous population than non-diabetics (Marples, 1965).

Severely ill patients may carry gram-negative organisms on their skin more often than do healthy people (Stratford *et al.*, 1968), but this was not shown by this group of well-controlled diabetics (Somerville & Lancaster-Smith, 1973). In fact they yielded gram-negatives less often than a healthy population—another unexpected twist in the tangled story of infections in diabetes.

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News and Notices

Announcement

The European Society for Dermatological Research and the Society for Investigative Dermatology will hold a joint meeting at the RAI International Congrescentrum in Amsterdam, Holland, 9–13 June 1975. The purpose of this meeting will be to serve as a forum for the presentation of original dermatological research papers. Luncheon workshops will be held each day as well as special lectures interspersed during the meeting. The program will be selected by a Joint Program Committee of the two sponsoring Societies. The deadline for mailing of abstracts is 3 January 1975. Twelve minutes will be allotted per paper. All presentations must be in English as simultaneous translation will not be provided. All program proposals must be submitted on the official forms which will be mailed to all members of both Societies. Non-members may obtain forms and further information from:

Dr Charles M. Lapière,
ESDR Secretary,
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Housing arrangements should be made through:
Organisatie Bureau Amsterdam, N.V.,
Postbus 7205,
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A registration fee will be assessed to cover the cost of the meeting hall and other expenses, but the amount has not yet been determined.

For both meetings a new concept in dissemination of scientific information will be introduced—'poster sessions'. Certain abstracts will be selected to be presented at these sessions in which authors display their material on 4 × 4 ft poster boards. These sessions will not conflict with the formal presentation of papers and will occupy only a portion of the total meeting time. It is hoped that these poster meetings will allow more investigators to present their data and will create an informal atmosphere for in-depth discussions (see *Science*, 28 June 1974, p. 1361). Abstracts for the poster session will be published in their entirety in the program and authors, at the time of submission of abstracts, can request their preferred method of presentation.

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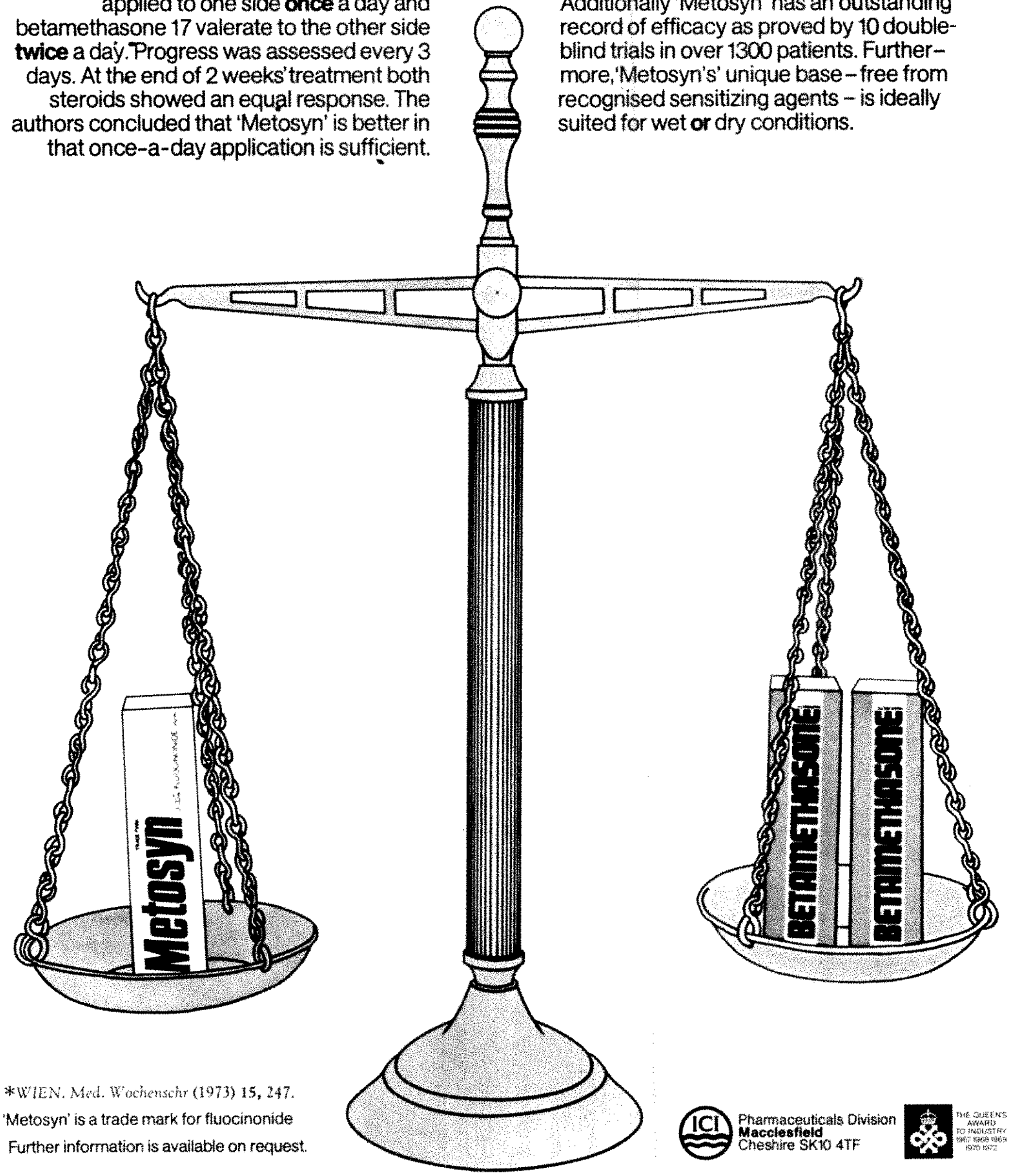

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The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

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Introduction

This volume consists of the proceedings of a conference, Steroids and the Skin, which was held in Edinburgh on 9 and 10 October 1975 and includes those parts of the discussion which were felt to amplify or add to the papers.

We would like to thank our Chairmen, Professor M.W.Greaves, Dr C.D.Calnan, Dr C.F.H. Vickers, Dr A.Lyell, Dr J.A.A.Hunter and Dr D.I.Williams as well as our contributors among whom we number, not only those who gave papers, but the many who took part in the discussions. To Dr C.G.Sparkes, Miss Pam Hawken and Mr David Kedgley, on whom most of the burden of organizing the meeting fell, and Mrs Eileen Weeks for her secretarial assistance both with the meeting and with the preparation of this volume, we are especially grateful.

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Steroid structure and steroid activity

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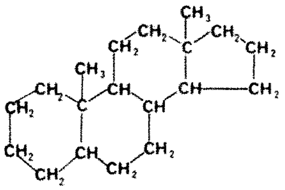
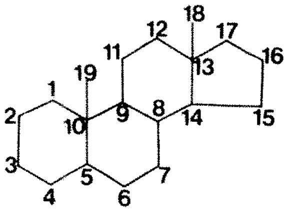
SUMMARY

The diverse biological effects associated with naturally occurring steroids are reviewed, with particular reference to the effect upon biological activity of changes in chemical structure. The motives for synthetic modification of the natural products are discussed and are illustrated by reference to the development of systemic and topical anti-inflammatory agents, with improved potency and reduced side-effects.

The name 'steroid' has tended to become identified with the relatively small group of anti-inflammatory compounds, based on cortisone, that has achieved prominence in the last twenty years or so. This, of course, is a misconception; the steroids comprise a very large group of natural products—widely distributed in both animals and plants—as well as their synthetic analogues.* They are remarkable as I shall try to show, for the diversity of their biological effects. In some instances, these effects are a reflection of the natural function of the compound; in others, the effect is pharmacological, rather than physiological.



(1)



(2)

The essentials of the steroid structure are shown in Formula 1: it is an array of seventeen carbon atoms in four fused rings—three of them six-membered, the other five-membered—with another two carbon atoms protruding from angular positions. This arrangement of carbon atoms is common to all but a few of the compounds that I shall be mentioning and, indeed, to the vast majority of compounds classified as steroids; the associated hydrogen atoms will vary from compound to compound, as substituents are introduced. The conventional representation of the steroid molecule and the method of numbering the carbon atoms are shown in Formula 2.

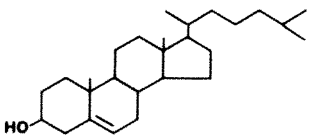
---

* Chevreul, in 1816, applied the name 'cholesterine' (Greek χολη, bile and στερεος, solid) to the crystalline compound isolated from human gall-stones. The name 'steroid' is now used generically for compounds of the same chemical class.

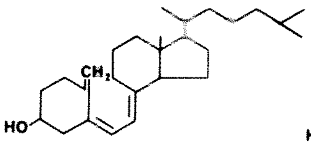
It should be noted that the structure has no elements of symmetry and that each of the 19 positions is, therefore, distinguishable from each of the others; hence, with a relatively few substituent groups permuted among these 19 positions, one can derive a very large number of different chemical compounds. The possibilities for variation are even greater than this: the framework of carbon atoms is three-dimensional, with ‘the front’ quite different from ‘the back’. For any given position in the rings, a monovalent substituent can be attached in the ‘α’-configuration, projecting towards the rear, or in the ‘β’-configuration, projecting towards the front of the molecule; these alternatives will be chemically and biologically distinct from one another.

Because the steroid skeleton is a rigid structure, even a small change in the position of a substituent will, very often, result in a large change of biological activity. This specificity is presumably a consequence of the necessity for the molecule to interact in a particular way with a highly structured protein receptor in order to exert its effect.

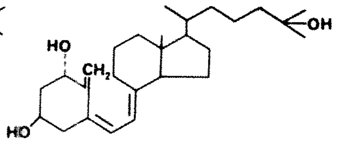
I shall start my survey with cholesterol (3), which is Nature's own point of entry into steroids proper. It will be seen that cholesterol has the steroid skeleton of Formula 1, together with a branched eight-carbon side-chain in the 17-position. In both animal and plant tissue, a very remarkable series of enzymic reactions builds up the twenty-seven carbon atoms of this compound, with the two carbon atoms of acetic acid as the fundamental building block. In turn, cholesterol is subjected to a variety of enzymic reactions to give most of the other naturally occurring steroids, and it is important for its central position in the biogenesis of this class of compound. Although it occurs widely in the lipid components of animal tissue and is a major component of such intrusions as gall-stones and atherosclerotic plaques, cholesterol itself would appear to function as a structural material rather than as a physiologically active compound.



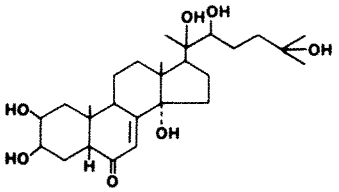
Cholesterol
(3)



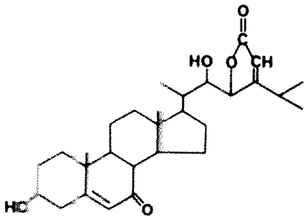
Cholecalciferol
(Vitamin D_3)
(4)



1α, 25-Dihydroxycholecalciferol
(5)



Ecdysterone
(6)

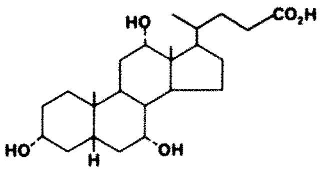


Antheridiol
(7)

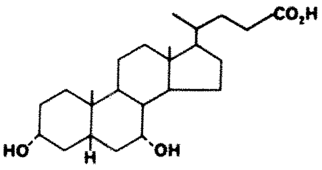
Vitamin D_3 (cholecalciferol: 4) is a close structural relative of cholesterol, with the unusual feature that one of the rings has been opened. It is now known that the vitamin is metabolized, first in the liver, then in the kidneys, to 1α,25-dihydroxycholecalciferol (5), in which two new oxygen atoms have been introduced; it is believed that it is this compound that is responsible for controlling absorption and transport of calcium (Holick & De Luca, 1974).

A very different sort of hormonal control is exerted by the ecdysones, a recently discovered group of compounds that control the moulting process in insects and crustaceans. Typical of the group is ecdysterone (6), in which the cholesterol skeleton is intact but has a number of oxygen atoms characteristically distributed around the molecule (Wyatt, 1972).

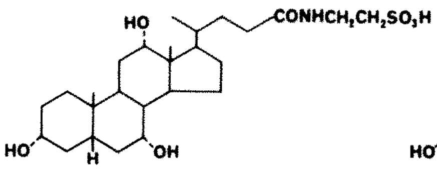
Yet another type of hormonal action is shown by a related compound, antheridiol (7), which acts as a sex hormone in the aquatic fungus, *Achlya bisexualis*; it is secreted by the female mycelium and induces formation of antheridial hyphae in the male strains of the plant (Raper, 1971). As will appear later, the steroids that act as sex hormones in higher animals are of a quite different type.



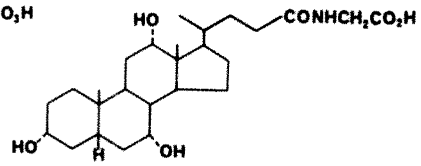
Cholic acid
(8)



Chenodeoxycholic acid
(9)



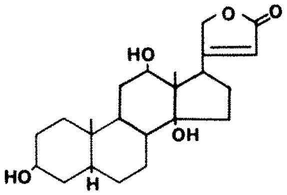
Taurocholic acid
(10)



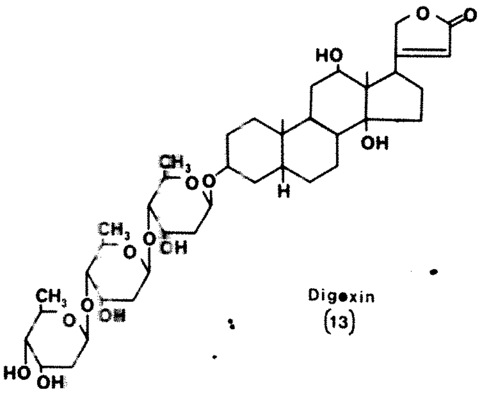
Glycocholic acid
(11)

A very important group of compounds, the bile acids, are derived from cholesterol by loss of three carbon atoms from the end of the chain, and introduction of oxygen atoms in various positions in the ring as well as at the end of the chain. The principal bile acids in man are cholic acid (8) and chenodeoxycholic acid (9). There are many more variations on this theme: the precise compounds and the ratios in which they occur vary from species to species, and there appears to be some evolutionary significance to this (Haslewood, 1967). The bile acids occur chiefly in conjugated form; for example, taurocholic acid (10) and glycocholic acid (11), in which cholic acid is combined with taurine and glycine respectively. The biological function of these conjugates depends upon their physical properties; they are detergent-like and they facilitate dispersion and absorption of fats and other lipids, including cholesterol. They do not seem to have any highly specific activity.

The shortening of the side-chain is taken a step further in the cardiac glycosides, a group of



Digoxigenin
(12)



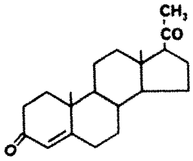
Digoxin
(13)

compounds of plant origin. Their function in the plant is obscure, but they are of great clinical importance for their tonic action on the failing heart. Again, they comprise a large series of closely related compounds, of which digoxin (13) is one of the few that are used in medical practice. To the usual steroid skeleton is now attached, at the 17-position, a branched chain of four carbon atoms, bridged by an oxygen atom to produce the lactone ring that is characteristic of this class; equally characteristic is the complex carbohydrate structure attached at the 3-position. This last feature is important for its effect upon the distribution characteristics of the drug but is not essential for its activity. Thus, digoxigenin (12), the steroid moiety of digoxin, itself has cardiotonic activity but it is not clinically useful because its action is very transient.

The next step in the loss of the cholesterol side-chain takes us to the pregnanes, in which a chain of only two carbon atoms is attached to the 17-position of the steroid nucleus. This is a series in which rather small modifications of substituent groups result in big changes in biological activity. The simplest of the series is progesterone (14), carrying ketonic oxygen atoms at positions 3 and 20. This hormone is secreted by the ovary and is essential, in combination with oestrogen, for the maintenance of pregnancy. Introduction of another oxygen atom, at the 21-position, confers a quite different type of activity; ability to control electrolyte balance, and, with it, water balance (mineralo-corticoid activity). Deoxycorticosterone (15) is an example of this type of structure; this compound is, indeed, formed in the adrenal cortex, but the circulating hormones are corticosterone (16) and aldosterone (17) which are derived from deoxycorticosterone by 11-mono- and 11, 18-di-oxygenation, respectively.

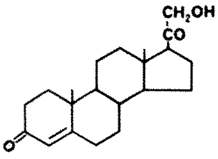
The introduction into corticosterone of yet another oxygen atom, this time in the 17-position, produces hydrocortisone (19). This compound retains a little of corticosterone's effect on mineral metabolism but a number of other effects are now found; they include ability to increase breakdown of protein and deposition of glycogen in the liver (glucocorticoid activity), and inhibition of the inflammatory and the immune response. These and other properties of hydrocortisone and its analogues are considered in much more detail in Dr Snell's paper. Cortisone (18) is readily interconvertible with hydrocortisone and shares its biological properties in most situations.

Finally, in the body's production of hormonally active steroids, it removes the side-chain altogether to produce the male and female sex hormones. Thus, testosterone (20) arises from progesterone (14) by loss of the two-carbon side-chain and introduction of an oxygen atom in its place. For the formation of the phenolic compounds, oestradiol (21) and oestrone (22), one of the original nineteen carbon atoms (see Formula 1) is lost.



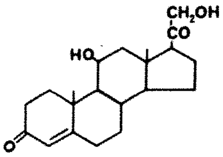
Progesterone

(14)



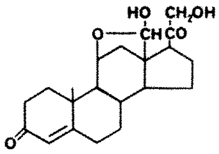
Deoxycorticosterone

(15)



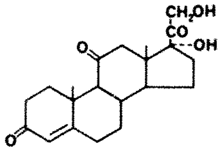
Corticosterone

(16)



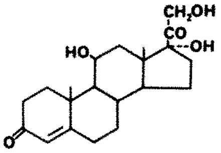
Aldosterone

(17)



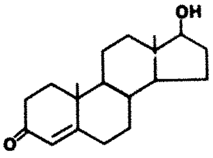
Cortisone

(18)



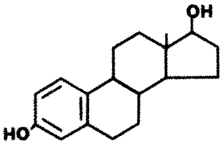
Hydrocortisone

(19)



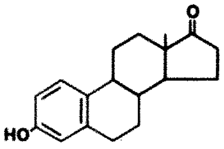
Testosterone

(20)



Oestradiol

(21)



Oestrone

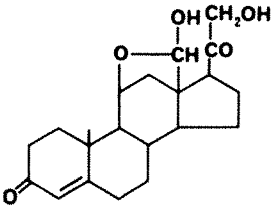
(22)

These are some of the more important ways in which the steroid skeleton is used, in Nature, as a template upon which are impressed more or less minor structural changes, often accompanied by major changes in biological activity. The list is far from being comprehensive; the steroidal alkaloid, malouetine, is a muscle-relaxant, while other naturally occurring steroids and their close relatives have anti-bacterial, anti-fungal, anti-amoebic and anti-tumour activity. With this rich variety of biological effects already available in naturally occurring steroids, it might be asked why the chemist bothers to look further. There are several reasons for his doing so, the more important of which are:

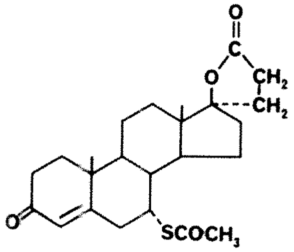
1. The steroid structure may be modified in the hope of finding yet further types of activity, unknown in the natural compounds; although this has led to some active compounds, none has found practical use, so far.

2. Inhibition of the action of a natural hormone may have therapeutic uses, and a competitive antagonist may be found among compounds structurally related to the hormone in question, presumably because they compete for active sites in the receptor. The outstanding example in the steroid series is the use of spironolactone (24) as a diuretic. Spironolactone is a competitive antagonist to the water-retaining compound, aldosterone (23), possibly because it mimics its structure (Kagawa, 1964).

3. A compound of known and useful activity may be modified in order to improve its pharmaco-



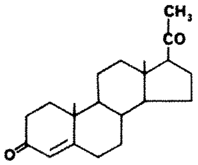
Aldosterone
(23)



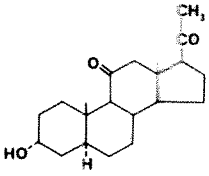
Spironolactone
(24)

kinetics. Thus, some of the steroid hormones are not absorbed from the gastrointestinal tract but relatively minor modification has left the activity unaffected while allowing the oral use of the drug. Prolongation of action may be desirable and there are fairly well-understood methods for achieving that end. Again, modification of physical properties may be necessary in order to allow absorption and action at a selected site, so improving the efficiency of use and reducing the likelihood of side-effects resulting from systemic administration.

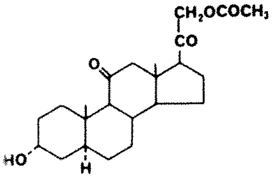
4. The chemist's most usual motive in undertaking structural modification of a naturally occurring steroid is to improve on its activity, qualitatively or quantitatively, or both. Increase in potency may be helpful, particularly if it reduces the cost of treatment, but it is not as important as the separation of activities. Most of the hormones exert a complex of effects and, for their therapeutic use, some of these actions may be unnecessary or positively harmful. Because of our ignorance of the detailed mode of action of most of these compounds it is not always easy to know, other than by trial and error, which actions are separable. None the less, a great deal has been achieved, largely by such empirical methods.



Progesterone
(25)



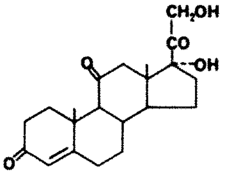
Alphaxolone
(26)

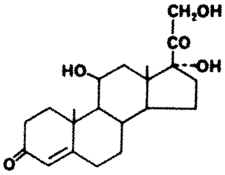


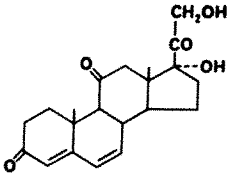
Alphadolone Acetate
(27)

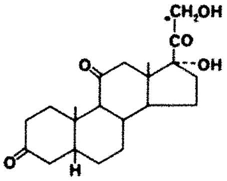
In such work, the aim is usually to emphasize the predominant action of the parent compound and to suppress the minor effects. There is at least one example of the successful application of the converse method. Progesterone (25) has a feeble hypnotic action in addition to its characteristic progestational effect. Modification of the structure has led to compounds in which the hypnotic activity has been greatly intensified and the hormonal activity eliminated; the intravenous anaesthetic, Althesin, is a composition containing two such compounds, alphaxolone (26) and alphadolone acetate (27) (Phillipps, 1975).

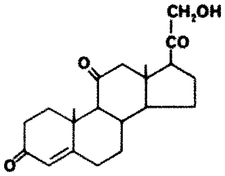
The development of the anti-inflammatory steroid drugs in use today serves to illustrate some of the principles that I have outlined. The announcement, by Hench and his colleagues in 1949, of the

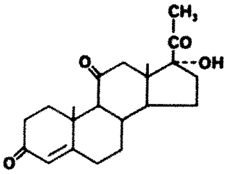

Cortisone
(28)


Hydrocortisone
(29)


6-Dehydrocortisone
(30)

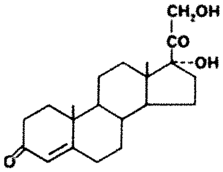

4,5β-Dihydrocortisone
(31)

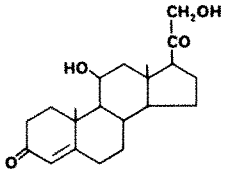

11-Dehydrocorticosterone
(32)

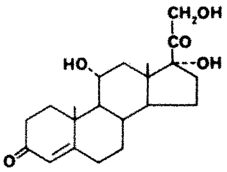

21-Deoxycortisone
(33)

effect of cortisone (28) in rheumatoid arthritis was followed by an explosive growth in research in steroid chemistry; this was largely directed towards the search for economic methods of synthesis of cortisone but some analogues of the hormone were synthesized and tested for anti-rheumatic activity. For a number of years the only other compound to display such activity was hydrocortisone (29) and it was early established that the two compounds are readily interconvertible in the body. Other changes, even changes that seemed quite trifling, had the effect of reducing the activity, often to the point of extinction. Examples include modification of cortisone (28); (a) by removal of two hydrogen atoms from positions 6 and 7 to give Compound 30, (b) by addition of two hydrogen atoms at positions 4 and 5 to give Compound 31, and (c) by removal of an oxygen atom from position 17 or 21 to give Compounds 32 and 33, respectively. Similar loss of activity resulted from modification of hydrocortisone (29); (a) by removal of an oxygen atom from position 11 or 17 to give Compounds 34 and 35 respectively, or (b) by simply changing the OH group at the 11 position from 'the front' to 'the back' of the molecule, to give 11-epihydrocortisone (36).

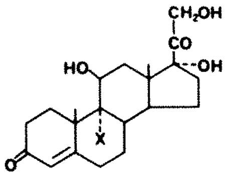
The situation was transformed in 1953 when Fried & Sabo synthesized the 9α-bromo- (39) and 9α-chloro-derivative (38) of hydrocortisone and found them to have glucocorticoid activity in laboratory animals: the chloro-compound was considerably more active than cortisone. The corresponding


Reichstein's Substance S
(34)


Corticosterone
(35)


11-Epihydrocortisone
(36)

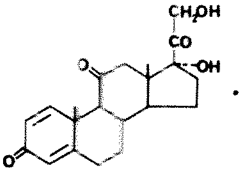
9α-fluoro-compound (37) proved to be still more potent: 8 times as active as hydrocortisone, itself, in an anti-inflammatory assay and 12·6 times as active in the liver–glycogen assay for glucocorticoid activity. Unfortunately, the fluorine atom was still more effective in increasing mineralocorticoid activity, so that 9α-fluoro-hydrocortisone could not be used in the clinic for its anti-inflammatory action. This work was important, none the less, for its demonstration that anti-inflammatory activity was not special to cortisone and hydrocortisone.



(37): X = F: 9α-fluorohydrocortisone

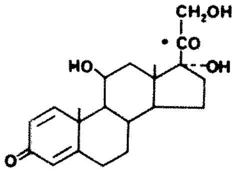
(38): X = Cl: 9α-chlorohydrocortisone

(39): X = Br: 9α-bromohydrocortisone



Prednisone

(40)

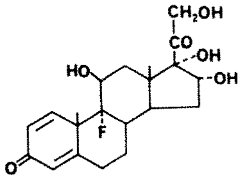


Prednisolone

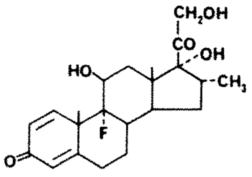
(41)

A second key discovery, announced by Herzog *et al.* in 1955, was that introduction of a 1,2-double bond into cortisone or hydrocortisone (i.e. removal of hydrogen atoms from positions 1 and 2) resulted in about a 4-fold increase in glucocorticoid and anti-inflammatory potency; in this instance, however, the mineralocorticoid effects of cortisone and hydrocortisone were reduced rather than increased. The new compounds, prednisone (40) and prednisolone (41), were the first synthetic analogues of the natural hormones to be used in the clinic for their anti-inflammatory properties and they are, of course, still widely employed. Another very important observation was that certain groups, particularly 16α-hydroxy, 16α-methyl and 16β-methyl, had the effect of nullifying the potentiation by 9α-fluorine of mineralocorticoid activity, without greatly affecting its potentiating effect on anti-inflammatory activity. The combination, then, of these features led to three compounds, triamcinolone (42), dexamethasone (43) and betamethasone (44), which remain among the most important of the compounds used as systemic anti-inflammatory agents. Other groups that enhance glucocorticoid and reduce mineralocorticoid effects include 6α-methyl and 6α-fluorine, which are found in methylprednisolone (45) and paramethasone acetate (46), respectively. These compounds (42–46) vary in clinical potency, from about equiactive with prednisolone to about 6 times this activity in the case of betamethasone and dexamethasone: they are all devoid of sodium-retaining properties. (The earlier work on modification of cortisone and hydrocortisone is very fully reviewed by Sarett, Patchett & Steelman, 1963.) However, there still remain side-effects that hamper the chronic use of all the systemic steroids currently available: they are discussed in Dr Snell's paper and I shall not detail them here. It remains to be seen whether some, at least, of these unwanted activities are separable from the anti-inflammatory action by new modifications of the molecule; this, certainly, remains a very important goal for further research.

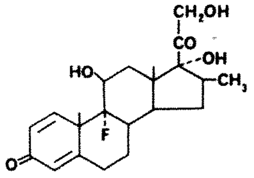
It has been known, since the early days of corticosteroid therapy, that many of the systemically active anti-inflammatory steroids are effective in the treatment of inflammatory conditions of the skin when they are applied topically. Some 15 years ago, it became apparent that certain structural modifications of these steroids resulted in compounds that were more effective than the parents. Since then, a number of new topical preparations have been marketed: Formulae 47–52 show the structures of the steroid components of a few of them. They vary somewhat in their potencies and in their



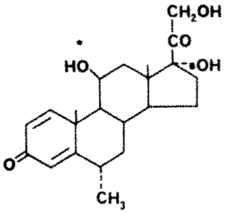
Triamcinolone
(42)



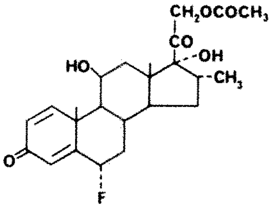
Dexamethasone
(43)



Betamethasone
(44)

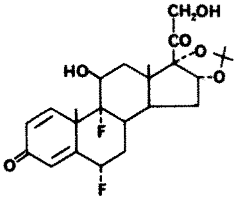


Methylprednisolone
(45)

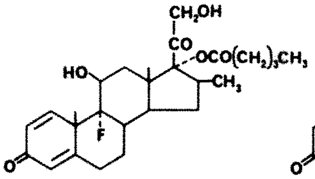


Paramethasone acetate
(46)

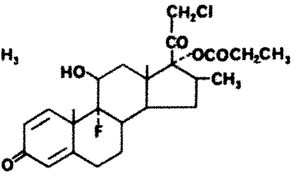
ability to cope with the severe dermatoses, clobetasol 17-propionate (49) probably being the most effective. It will be seen that they all contain many of the features that I have already described as conducing to high systemic activity and, generally speaking, they do show such activity. However, they differ from the usual systemic compounds in having been modified in ways that will tend to increase lipophilicity by removal or masking of hydroxyl groups or by introduction of long carbon side-chains, or both. Thus, in compound (47) hydroxyl groups at positions 16 and 17 are masked in an acetonide grouping, while in Compounds 48–51 a hydroxyl group at position 17 or 21 is masked by



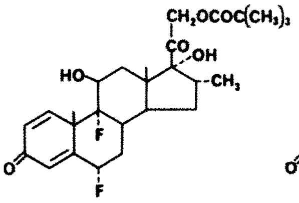
Fluocinolone acetonide
(47)



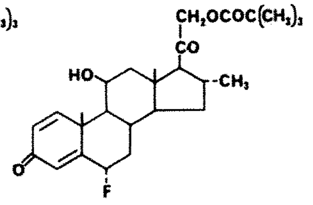
Betamethasone 17-valerate
(48)



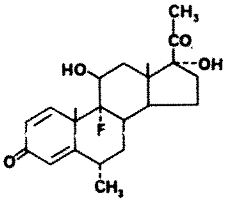
Clobetasol 17-propionate
(49)



Flumethasone 21-pivalate
(50)

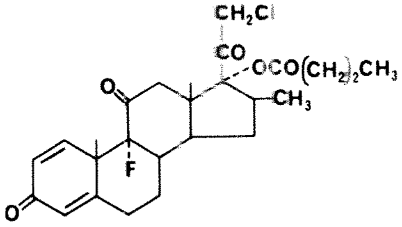


Fluocortolone 21-pivalate
(51)



Fluorometholone
(52)

esterification with a fatty acid. Additionally, Compound 49 has a chlorine atom in place of a 21-hydroxyl group and Compounds 51 and 52 have hydrogen atoms at positions 17 and 21, respectively, rather than hydroxyl groups. It seems likely that these modifications, by increasing solubility in fat, relative to that in water, have the effect of improving the availability of the steroid at the site of action in the skin; correct matching of the pharmaceutical vehicle to the steroid can be very important in optimizing the effect (Popper & Watnick, 1974; Phillipps, 1976). There remains the possibility of absorption into the system, with consequent unwanted side-effects, but there are indications that topical and systemic activity do not inevitably run parallel and that it may be possible to reduce still further the slight danger of systemic effects in topical steroid therapy. Clobetasone butyrate (53) appears to show such a separation of topical and systemic effects and, in addition, to be less prone to cause skin atrophy than are other steroids of similar potency; this will be discussed in detail by other contributors to this volume. An unusual structural feature of clobetasone butyrate is the ketone group at position 11; this contrasts with the 11β-hydroxy-group that is usually associated with topically active anti-inflammatory steroids (cf. Formulae 47–52).



Clobetasone butyrate

(53)

The significance of steroid structure in relation to activity is still far from clear. Ultimately, the steroid, in order to exert its effect, must interact with some receptor in a very specific way and structural changes that interfere with its ability to do so will cause loss of activity. Among such factors might be a significant change in the overall shape of the molecule, or a change of substituent at an important binding site. Conversely, the affinity between steroid and receptor may be enhanced by more subtle changes. It has, for example, recently been noted that certain substituents that increase anti-inflammatory potency have a similar effect on the shape of the steroid molecule (Weeks, Duax & Wolff, 1973). Again, one effect of the 9α-fluorine atom may be to increase the ability of the 11β-hydroxyl group to bond with electron-donor groups in the receptor (Fried & Borman, 1958). There is evidence that some of the groups that potentiate anti-inflammatory activity reduce the rate of metabolic destruction of the compound (Sarett *et al.*, 1963). Yet again, a theory that may account for the effect of certain substituents, including 9α-fluorine, is that, in the equilibrium between 11β-hydroxy and 11-ketone, they favour the former, which is thought to be the form in which the anti-inflammatory steroids exert their effect (Bush & Mahesh, 1964).

A great deal has yet to be done before the detailed mechanism of action of many of these compounds can be understood. In the meanwhile, we can, by more empirical means, gain an understanding of the significance, for potency, for selectivity, for distribution and metabolism, of particular sites in the molecule and of particular types of structural change at those sites. In this way, still further refinements of steroid structure and still greater specificity of action should be attainable.

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DISCUSSION

PROFESSOR M.W.GREAVES (LONDON): Senior colleagues who were around at the time tell me that when steroids were first introduced to clinical dermatology, there was great disappointment that cortisone itself did not work when applied to the skin. You mentioned that most body tissues contain enzymes which are capable of converting the cortisone to hydrocortisone, and the skin is certainly one of these. It has always puzzled me that no anti-inflammatory action was found when cortisone was applied to the skin.

DR J.ELKS: It is an oddity, and it is a fact that nearly all of the compounds that are in use have an 11β-hydroxy rather than an 11-ketone. It is a feature of clobetasone 17-butyrate (53) that this has an 11-ketone group, as has cortisone, rather than the 11-hydroxy group. There are a certain number of other activity-enhancing groups in clobetasone butyrate that might overcome whatever deleterious effect the ketone may have.

PROFESSOR M.W.GREAVES (LONDON): Is the hydroxy group really enhancing purely because of potency or of penetration?

DR J.ELKS: I think it certainly increases potency. The 11β-hydroxy group has the effect of turning the inactive Reichstein's Substance (34) into the very active hydrocortisone (29); this potentiating effect applies to the systemic as well as to the topical activity. It is more than a simple matter of affecting penetration.

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The pharmacological properties of corticosteroids in relation to clinical efficacy

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SUMMARY

The pharmacological properties of cortisol (and cortisol-like drugs) are divided into several distinct activities according to the type and cellular site of action of the hormone. To each of these discrete properties is allotted the separate clinical beneficial responses and the adverse effects that probably arise from them. For any one patient only one, or two, of these pharmacological properties are desirable and the remainder are undesirable. Therapy aims to separate the wanted from unwanted effects and this can be attempted in three ways: firstly, by adjustment of dose, frequency and duration of corticosteroid administration; secondly by delivery of the drug directly only to the site of disease—if this is possible; and thirdly, by using analogues which have been synthesized with 'tailor-made' selective actions. Synthetic analogues have not yet offered much selectivity of action of value for systemic use, but for local use (as on the skin and in the respiratory tract) such analogues have provided a considerable therapeutic advance.

It is suggested that the several distinctive pharmacological properties of cortisol (as well as the existence of other steroids closely related chemically but with widely different biological activities, such as progestagens and anaesthetics) offer hope for the synthesis of cortisol-like compounds with much greater selectivity in systemic action.

Among the normal substances produced by the body, hydrocortisone (cortisol) is unique in having a powerful ability to alleviate a wide range of diseases. This extraordinary property results only when administration of the substance raises its circulating and tissue concentration above that pertaining normally, when the effect becomes pharmacological rather than physiological. Until we discover precisely how cortisol works in disease we are unlikely to know to what extent, if at all, these two roles are related. Such puzzles, however, need not hold up progress in the better therapeutic use of cortisol-like steroids. It was thought to be of interest in this connection to examine the pharmacological properties of cortisol and its analogues to determine those which might be due to different mechanisms—at least at the cellular level. By relating to these discrete pharmacological properties both their specific therapeutic responses, and the associated adverse effects, it is hoped to indicate how cortisol-like hormones may be better used clinically. For each pharmacological property of cortisol will be given a brief definition of the diagnostic tests used (in animals unless stated otherwise), the probable cellular site of action, the possible clinical benefits (as opposed to the physiological actions, which are ignored) and the adverse effects that can be assigned to it. Replacement therapy for adrenal insufficiency is ignored as this merely utilizes physiological properties.

Mineralocorticoid activity

This property relates to the metabolism of water and electrolytes, with their secondary effects on blood pressure, and results from action on the renal tubules. It is assessed by the degree of sodium retention and potassium loss induced by a compound. The only therapeutic benefit that might result from this activity of cortisol is its controversial use in various states of 'shock', where induced retention of extracellular fluid and blood volume expansion may assist maintenance of blood pressure and the arterial circulation.

As would be predicted, the adverse clinical effects of mineralocorticoid activity are oedema resulting from retention of sodium and water, hypokalaemia from potassium loss, and raised blood pressure.

Glucocorticoid activity

Metabolic effects on glucose, protein and fat are the hallmarks of corticosteroids (synthetic or natural) with other cortisol-like properties—hence the term 'glucocorticoids'. Glucose metabolism is widely affected by mobilization of muscle glycogen, increased storage of liver glycogen, glucose formation from amino acids and its impaired peripheral uptake from antagonism of insulin. These, often opposing, effects on blood sugar are further complicated by secondary responses such as increased insulin secretion, so that this property is best assayed by the degree of glycogen deposition in the liver. Protein catabolism is increased to give amino acid mobilization with a resulting negative nitrogen balance. The target cells for this activity must be very many and varied, worthy of the description indiscriminate.

Very few clinical benefits arise directly from these metabolic effects. The increase of appetite and body weight which result from corticosteroid therapy may arise in this way, but, although often acceptable as a secondary benefit, they would not alone be a therapeutic use. Patients with a combination of diabetes mellitus and adrenal insufficiency are highly sensitive to insulin and have wide swings in blood sugar. Cortisol therapy stabilizes blood sugar by decreasing sensitivity to insulin and makes the diabetes easier to manage. Glucocorticoids are sometimes used temporarily to antagonize excess endogenous insulin from pancreatic islet cell tumours.

In a veterinary condition, bovine ketosis, this property of steroids is used therapeutically. In early lactation, carbohydrate starvation relative to protein intake causes severe hypoglycaemia and ketosis, which is promptly reversed by parenteral corticosteroid administration.

The paucity of clinical uses of the metabolic properties contrasts with the long list of their adverse effects, such as the appearance of Cushingism, diabetes mellitus, loss of muscle and bone tissue, and pathological fractures. They may also account, at least partly, for myopathy, vascular thromboses and cataract.

At present these metabolic properties remain strongly linked to therapeutic effectiveness in inflammatory and allergic disease. It has been postulated that corticosteroids act by impairing glucose uptake by cells and producing a general brake on their activity. If this were the mode of action it might explain the link between therapeutic and metabolic activities, otherwise there is no obvious association between them.

Anti-inflammatory response

The anti-inflammatory activity of cortisol is said to be 'non-specific' as it is exerted in widely different types of inflammation (infective, allergic, physical) and at different stages. This activity is assayed in animal models with 'non-specific' irritant stimuli, such as injections into the tissues of carageenin, croton oil and turpentine oil, with measurement of the resulting inflammatory lesion. In inflammation, polymorphs and monocytes first stick (marginate) to the walls of minute vessels then penetrate them and migrate to the diseased area (chemotaxis). Dilatation of these vessels and an increase of their permeability gives rise to tissue swelling. Glucocorticoids reverse all these features of inflammation. Prevention of margination and chemotaxis appears to be a direct effect on leukocytes but it is not clear if the response of the minute vessels is primary, or secondary to some other action. In natural inflammation the presence of leukocytes is probably necessary for the response which is mediated by several endogenous substances of both cellular and humoral origin (Fig. 1). There are also important inter-relations with other complex chain (or cascade) reactions such as the kinin system, the clotting system, and activation of complement. All these three humoral systems may be activated by the Hageman Factor (Factor XII) which itself is activated by surface contact of various kinds. The plasmin system and platelets may also be involved.

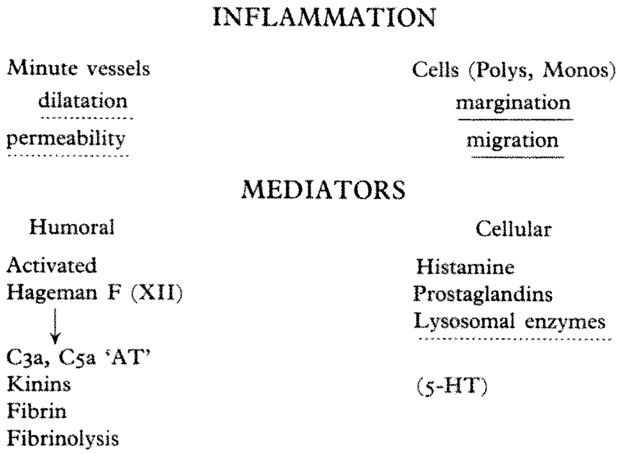

FIGURE 1. The main features and mediators of inflammation. .... a possible, and ——— a probable site of action of cortisol.

Thus 'non-specific' inflammation may have highly specific pathways from its complicated relations with other systems. There appears to be no evidence that cortisol in therapeutic concentrations consistently and directly influences the intermediate events of inflammation. There have been contrasting claims of suppression *in vitro* of phagocytosis and of lysosomal enzyme release from cells by corticosteroids in enormous doses, contradicted by other work in which, in therapeutic amounts, no such effects can be shown. Cortisol in high concentration suppresses endogenous pyrogen formation by human leukocytes *in vitro*, but this effect is also exerted by oestrogen and is absent with rabbit leukocytes so its significance is not clear. A key action of corticosteroids may be denial of access of cells to sites of inflammation by blocking margination and chemotaxis. The site of action appears to be highly localized to these inflammatory cells and to be sufficiently distinctive in type to differentiate this property from the others considered.

The anti-inflammatory property of corticosteroids provides their main therapeutic use in a wide range of acute and chronic inflammatory diseases (including most steroid-responsive dermatoses), irrespective of aetiology. Some apparently unrelated clinical benefits may also be mediated by the anti-inflammatory effect, such as the reduction of cerebral oedema due to tumours and infarcts, and improvement of acute heart block due to myocardial infarction.

The adverse results of this pharmacological property are decreased resistance to infections—

especially those combated by polymorphs and monocytes and those which require localization of the infection by thrombi, such as bacterial disease.

Suppression of specific immunity

Immune reactions which are harmful to the host (rather than protective) have been usefully classified by Gell and Coombs into four groups of allergy, although in disease there may well be overlap and variations in these types. Reactions mediated by antibody (derived from B lymphocytes) are shown in Fig. 2 and comprise Type I (anaphylactic, with IgE antibody bound to mast cells and basophils), Type II (with antigen bound to cells) or Type III (with circulating antigen–antibody complexes). Cell-mediated reactions are categorized as Type IV and are mediated by T lymphocytes. Inflammation is an integral part of all these responses, so that anti-inflammatory effects of cortisol are also inevitably involved in the clinical response as a whole.

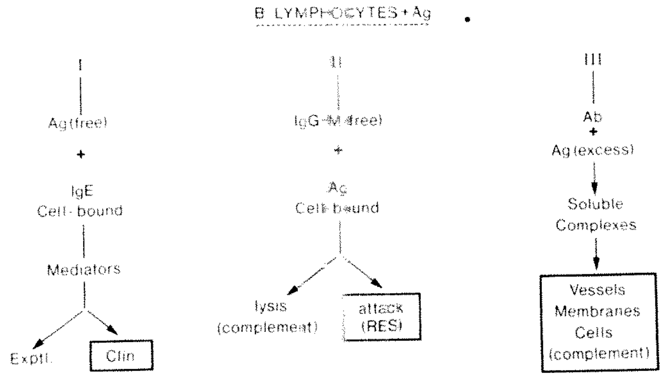

FIGURE 2. Simplified description of Types I, II and III antibody mediated allergy. ——— a probable, and ········ a weak site of action of cortisol.

Numerous animal models are available for the study of all these types of specific immunity: anaphylactic responses (*in vivo* and *in vitro*), active or passive sensitization with antibody, tissue transplants and induced allergic reactions in brain, joint and other tissue.

Lymphoid cells occupy a key place in immunity and several different effects are exerted on them by cortisol. The division of immature lymphoid cells is inhibited and a direct toxic effect occurs. Additionally sequestration at various sites in the circulatory system appears to account for many of the acute and profound changes in the circulating white cell population (lymphocytes, monocytes and eosinophils). These events tend to reduce access of effector T cells to target cells. Similarly, reduction of the B cell population may reduce antibody production but high and prolonged dosage of cortisol is needed for this effect. In experimental Type I reactions, such as with prick tests or bronchial challenge with antigen, cortisol has no certain action at all. Yet, in clinical allergy thought to be based on this Type I reaction, cortisol is effective—perhaps solely by its anti-inflammatory properties. In Type II reactions, cortisol does not affect intravascular lysis of immune red cells by complement but may block the destruction and phagocytosis of antigen-coated cells by the reticulo-endothelial system (RES) in some 'autoimmune' diseases. In Type III reactions, steroids block the noxious action of antigen–antibody complexes on target cells, which are usually polymorphs, and vascular endothelium. Hence, in these three types of allergy cortisol acts primarily on phagocytes, either fixed (RES) or circulating (polymorphs), an effect akin to its 'non-specific' anti-inflammatory property.

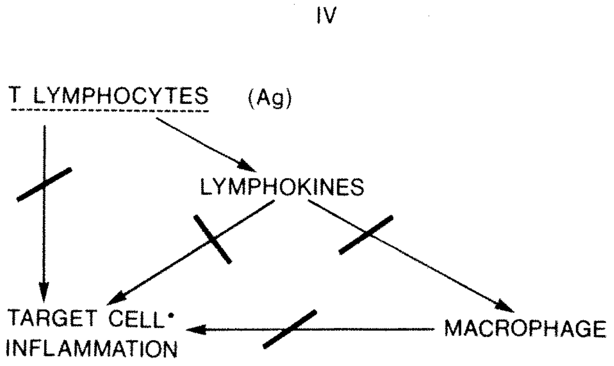

FIGURE 3. Simplified scheme of cell-mediated allergy (Type IV). ▬ a probable and ······ a weak site of action of cortisol.

Type IV reactions are suppressed at several points as shown in Fig. 3. There is inhibition of the action of toxic products (lymphokines) released from effector (sensitized) T lymphocytes on target cells and macrophages, and corticosteroids may also prevent access of both T cells and macrophages to, and their attacking actions on, target cells. This combination of actions seems so distinct from other cortisol effects as to form a separate immuno-suppressive property.

Clinically, suppression of specific immunity has many uses, including in all the allergies. Allergic contact dermatitis seems to be one common skin disease based on a Type IV cell-mediated reaction, and cutaneous allergic vasculitis may be a Type III response.

The side effects of this property result from reduction of cell-mediated immunity to protective vaccines and pathogens, including especially viral, fungal and chronic bacterial infections (such as mycobacteria).

Tissue involution

Corticosteroids inhibit DNA synthesis in a wide variety of tissues but there are three particular target cells or tissues which involute notably: lymphoid cells, skin and fibroblasts.

1. *Lymphoid.* The cytolysis and inhibition of division of premature lymphoid cells is primarily an involutional effect. The thymus shrinks considerably under the influence of corticosteroids and this forms a convenient method of measuring the lympholytic property. Mature T cells do not seem to be much affected and immunocompetence of the thymus as a whole is not reduced even though only 5% of the cell population may remain. The effectiveness of cortisol treatment of lymphatic leukaemia, lymphomas and myeloma appears to be a result of this property alone. As noted previously, this property may also partly explain immuno-suppressive functions in cell-mediated allergy.

The effect on lymphocytes does not appear to progress to total lymphopoenia with the consequences that would result, although it may contribute to the diminished resistance to infection.

2. *Skin.* Animal skin differs anatomically and often pharmacologically from human skin, so that information relevant to skin thinning in man is best derived from man. Other papers in this volume describe this property in detail. Depression of epidermal cell turnover would seem to be helpful in the treatment of some proliferative skin disorders such as psoriasis. However, evidence presented both here and elsewhere suggests that the clinical response of skin to corticosteroids is largely anti-inflammatory and is not necessarily dependent on skin thinning.

3. *Fibroblasts*. Involution of fibroblasts produces secondary depression of collagen production in skin. This effect is used clinically in the prevention and treatment of excess scar tissue and cheloids in skin healing.

Important and numerous adverse effects of these involutional properties of corticosteroids on skin can result from systemic or topical use and include skin thinning, striae, bruising, telangiectasia and delayed wound healing. Tissue involution of this kind may also contribute to two complications, myopathy and osteoporosis.

Peptic ulceration can result from long-term use of corticosteroid; the mechanism is unknown but involution of gastric mucosal cells could be involved.

Endocrine effects

Suppression of the hypothalamus by corticosteroids causes pituitary–adrenal suppression and, depending on dose and duration, of growth hormone release. These effects can be measured in animals (by the ether-stress test) and man (as described elsewhere in this volume). Clinical use for these properties has not emerged apart possibly from suppression of certain states of adrenal hyperplasia and as a diagnostic test for Cushing's syndrome. Important adverse effects such as adrenal suppression and unresponsiveness and growth retardation can result from these properties.

Glucocorticoids probably play a role in normal parturition. Fetal cortisol at term passes into the maternal circulation and in some species is associated with a sudden reversal of oestrogen and progesterone production and their blood levels, together with prostaglandin release. These changes seem to induce uterine contractions and, both in animals and man, injected glucocorticoids induce labour at or near term. The precise pharmacological mechanism of this reaction has not been identified.

One odd pharmacological property associated with topical steroids is a progestational effect in animals. This varies in intensity with different compounds; the most potent appears to be fluocinolone acetonide which has about 50 times greater activity (weight for weight) than progesterone itself in the response of rabbit or rat. The mechanism is unknown, but has no obvious connection with other properties.

This progestational effect appears to have neither clinical use nor adverse effects but as is shown below, progestational agents have immunosuppressive properties.

Miscellaneous properties

Important cerebral or psychic properties of cortisol include depression, insomnia, euphoria, dependence, mania and psychosis. These effects presumably result from a direct action of drug on brain cells but there does not appear to be an animal model for their study. These symptoms serve no useful purpose therapeutically but they can form major adverse effects.

One cellular property much studied is enzyme induction, such as tryptophan pyrrolase and tyrosine amino transferase by liver cells, and this has revealed much about the intracellular actions of corticosteroids. Liver may not be typical of all cells, but this work appears to show that corticosteroids pass freely into cells where they bind with a specific receptor in the cytoplasm. This complex then migrates, under temperature-dependence, into the nucleus where it reacts with DNA. From the gene template, messenger (m) RNA is formed, which passes into cytoplasm to ribosomes. Here, from the mRNA template, new protein is formed, thus revealing a previously unexpressed cellular activity. This offers a satisfactory explanation for enzyme induction where the protein produced is readily identified as specific enzyme, but for the numerous other cellular effects of corticosteroids demonstration of specific effector protein is awaited.

One clinical use that may be accounted for in this way is the prevention of respiratory distress syndrome in premature babies. Glucocorticoids induce the formation of pulmonary surfactant in immature lung, the absence of which excites the respiratory disorder. There are probably other examples of the maturation of tissue *in utero* being dependent on cortisol.

Liver cell enzyme induction by corticosteroids increases the rate of metabolism of other drugs and lessens their effectiveness, so forming an adverse effect.

Differential pharmacological effects of steroids

The aim of corticosteroid therapy is to produce the required clinical benefit coupled with the least possible degree of side effects. Application of the drug directly, only to the site of disease, is one way of achieving this ideal; the posological approach is another—using a dose and frequency that permit the desired effect and minimize those that are undesirable; a third is to use a compound that selectively differentiates wanted from unwanted effects. To examine how far this third possibility has been realized, the approximate range of properties possessed by some steroids in clinical use has been illustrated (Fig. 4). Cortisol is shown to have some mineralocorticoid activity which, apart from replacement therapy, offers no clinical advantage over the more potent synthetic analogues. The synthetic glucocorticoid compounds (used systemically) have largely eliminated mineralocorticoid activity, and some even cause diuresis of sodium and water. Deoxycorticosterone and fludrocortisone have predominantly, and aldosterone has virtually only, mineralocorticoid activity. Compounds with side-chain esters have been synthesized which have greatly enhanced topical activity, and these present a major advantage when used topically in a wide range of diseases of the skin, eye and respiratory tract. Further enhancement of topical relative to systemic activity, as in clobetasone butyrate, has resulted in a compound which appears to have also a decreased potential to cause involution of epidermis and perhaps other cells. Is it possible to dissociate further any of the wide range of 'glucocorticoid' effects? The differences in mode and site of action of some of the properties of cortisol discussed here seem sufficient to offer hope that this can be achieved.

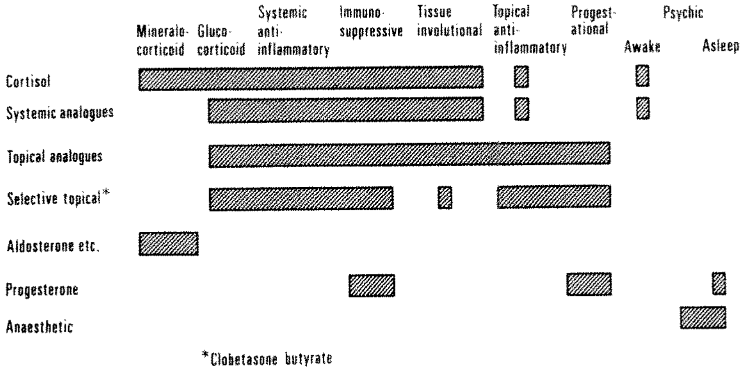

FIGURE 4. The pharmacological properties, indicated approximately, of some steroids in clinical use.

When steroids with no (or minimal) glucocortocoid activity are considered, very wide differences in pharmacological properties are found. Aldosterone has already been mentioned. Progestagens have immunosuppressive properties that in one animal model are greater than cortisol, but are without anti-inflammatory effects. Miliary eczema of cats, a seasonal inflammatory dermatosis that may be an allergy secondary to flea infestation, responds well to systemic progestagen therapy but less well to cortisol. It is of interest that steroid analogues with topical activity also have progestational properties in animal tests—presumably because their chemical configuration is suited to progesterone cell receptors. Although glucocorticoids induce euphoria, progesterone was noted by Selye to induce sleep, and this property was exploited by chemical synthesis to design an intravenous steroid anaesthetic. More recent research has further improved the properties of steroid anaesthetics to most potent products with the desired qualities of speed and duration of action and safety, but without endocrine effects. One virtue of steroid molecules as a group is their very low degree of chemical toxicity; side-effects appear to be related only to biological effects.

These examples of steroids with widely varying properties seem to offer further hope that the spectrum of 'glucocorticoid' effects can be broken up with new compounds.

This approach has been speculative with numerous assumptions, but it will have been justified if it has provided insight into the clinical use of corticosteroids and encouragment for further chemical research and development of them.

DISCUSSION

DR J.L.BURTON (BRISTOL): You pointed out the large degree of overlap between anti-inflammatory effect and immunosuppressive effect, but you did not specify what tests are best for measuring immunosuppression when developing new compounds.

DR E.S.SNELL: The problem here is that, apart from the vasoconstriction test in humans, one is always using animals. There is a variety of tests in the rat, adjuvant arthritis and allergic encephalomyelitis for instance and the skin of guinea-pigs can be sensitized with various chemicals to produce a model of contact sensitivity. One can do a wide range of things using passive anaphylaxis. The problem of selecting a test which will be most relevant to the human animal remains.

DR A.JARRETT (LONDON): What is your opinion of membrane stabilization as a mode of action?

DR E.S.SNELL: I think this membrane stabilizing effect has been grossly exaggerated, and workers in this field seem to pay much less attention to that than to other properties. It was used by Weissmann and colleagues as a way of explaining all the actions of steroids, but they were using very high doses and using only liver cells which may be atypical cells. I believe they were asked at one meeting, in response to a question such as this, if they had used any other common detergents. Dr Elks mentioned the detergent properties of some of these steroids, and when you get to the sort of supra-therapeutic doses being used in their experiments, they are producing physical effects which have nothing to do with the biological activity.

PROFESSOR M.W.GREAVES (LONDON): I agree with you that steroids acting by the stabilizing of membranes has been overrated. Telling evidence has been the work of Chayen and Bitensky. They have shown that hydrocortisone does stabilize the lysosomal membrane and inhibit the release of lysosomal enzymes *in vitro*. So does cortisone acetate *in vitro* and it has no corticosteroid activity whatever.

I disagree with the point about the glucocorticoid steroids not inhibiting the Type I reaction, the histamine reaction, *in vitro*. This story got about originally because when steroids were first used *in vitro*, cortisone acetate was the compound studied on Type I reactions, and it did not produce any inhibition of histamine release, presumably because it does not have corticosteroid activity. But if you look at the more potent cortisol-type steroids, you can show evidence of inhibition of histamine release *in vitro*.

DR E.S.SNELL: I do not think that is so if you are talking about therapeutic doses. You can take a patient who is having therapeutic doses of steroids and test him with some of these so-called Type I challenges and he still responds. I am sure that *in vitro* you can use a concentration sufficient to block the response, but in the clinical situation we have to come back to the right subject, which is man, and to the right dose which must be the therapeutic one; then I think it still remains the case that steroids do not block those responses, whereas they do block the clinical effects which are thought to be the result of the response. Therefore, either Type I response does not really happen clinically, or it is associated with something else.

Dr G.Holti (Newcastle): I think the reason asthma responds to corticosteroids, while the IgE Type I mediated reaction does not, is that asthma is a mixture of Type I and Type III.

Dr P.A.Dufton (Liverpool): The topic of drug interaction is fashionable at present. Are there any groups of drugs which we may be prescribing for patients which are interfering with the required steroid effect or inhibiting it?

Dr E.S.Snell: I mentioned the induction of liver enzymes by steroids. Other drugs especially barbiturates induce liver enzymes and increase the inactivating power of the liver on steroids, so that patients on barbiturates have lower blood levels. The reverse situation can result, not through drugs but when the liver function is impaired by hepatitis or other disease. The inactivation of cortisol is decreased and the blood levels are much higher. It is interesting that Hench, long before the use of cortisol, noticed there were two factors which improved rheumatoid arthritis: one was pregnancy and the other jaundice, and there was speculation whether these in some way might be concerned with the relief of rheumatoid arthritits.

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Acute tolerance to effects of topical glucocorticosteroids

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SUMMARY

Tachyphylaxis (acute tolerance) to two important actions of topical glucocorticosteroids is demonstrated. In humans, topically active glucocorticoids applied to the skin cause vasoconstriction initially but this response fades with successive applications. In hairless mouse epidermis, inhibition of DNA synthesis and mitosis is caused by one application of a glucocorticoid but with repeated administration of the drug this effect is lost. The degree of inhibition of cell division appears to be similar 30 and 54 h after one application of steroid to that following three or five applications during such a time period. These findings suggest that an optimum therapeutic approach to the use of these agents exists.

Tachyphylaxis (acute tolerance) is the rapid diminution of a pharmacological response with repeated administration of a drug (Goldstein, Aronow & Kalman, 1974) and occurs in particular with sympathomimetic amines. Originally this was thought to be due to occupancy or saturation of drug receptors (Winder, Anderson & Parke, 1948) thus preventing further effect of the drug but, based on work with indirectly acting sympathomimetic amines such as ephedrine, tyramine and amphetamine, the loss of effect was attributed to the depletion of noradrenaline stores so that further challenge would liberate increasingly small amounts of noradrenaline (Axelrod *et al.*, 1962).

The occurrence of tachyphylaxis to repeated applications of topical corticosteroids has not previously been recognized and is now described with two important effects of these drugs. Vasoconstriction, which is visible shortly after a corticosteroid is applied and which lasts for several hours, is a fundamental property of all the potent topical corticosteroids in clinical use. The relationship of this phenomenon to their therapeutic action is unknown but the McKenzie Stoughton vasoconstrictor test (1962) has been the standard bioassay system for screening for new topical corticosteroids. Indeed two of the most frequently prescribed topical corticosteroids, betamethasone valerate and fluocinolone acetonide acetate were discovered in this way. It appears that vasoconstrictor activity and therapeutic efficiency are closely related. The most potent topical corticosteroid at present available in this country, clobetasol propionate, has approximately 19 times the activity of fluocinolone acetonide and 6 times that of betamethasone valerate in vasoconstrictor studies (Sparkes & Wilson, 1974).

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Using the vasoconstrictor assay system, tachyphylaxis will be shown to occur with all the fluorinated corticosteroids tested which include triamcinolone acetonide, betamethasone valerate, fluocinolone acetonide and fluocinolone acetonide acetate (fluocinonide). Thus vasoconstriction occurs initially but with subsequent applications the blanching effect fades until it is no longer elicitable.

The vasoconstrictor assay has proved to be a reliable reproducible test although it is only a visual assessment. The phenomenon of tachyphylaxis was investigated further using an animal system and the effect of repeated applications of corticosteroids on epidermopoiesis was studied. Topically applied glucocorticosteroids are known to inhibit mitosis in normal human skin (Fisher & Maibach, 1971) and in psoriasis (Fry & McMinn, 1968; Baxter & Stoughton, 1970). Many believe that the rationale of therapy of this hyper-proliferative disease is to slow down this process by inhibiting DNA synthesis and mitosis, and that topical steroids exert their therapeutic activity in this manner. It will be demonstrated that tachyphylaxis also occurs to these chemotherapeutic properties of topical steroids both in a proliferating epidermal cell system and in normal epidermis. These findings may have important therapeutic implications.

VASOCONSTRICTION STUDIES

Material and methods

Using an Eppendorf pipette, 0·01 ml of steroid was applied to the volar aspect of normal forearm skin by one investigator, and then re-applied to the same site at various times. No guards or occlusive dressings were used and the subjects followed their normal routine for clothing and bathing. Ten subjects were investigated and the blanching grades are the average of twenty applications of steroid since both forearms were used in each subject. The vasoconstriction responses were read at certain times and graded visually by one independent trained observer: where 0 is no blanching; 1 just detectable blanching; 2 definite but not maximum; and 3 maximum blanching (Stoughton, 1972).

Experiment 1

Steroids investigated: 0·1% and 0·5% triamcinolone acetonide in N, N-dimethylacetamide (DMAC).

Application times: 3 times daily, *viz* at 0, 4, 8, 24, 28, 32, 48, 52, 56, 72 and 76 h followed by a rest period until 168 h, when a final application was made.

Reading times: As above and also at 80, 96, 172, 176 and 192 h.

Experiment 2

Steroid investigated: 0·05% fluocinonide (Metosyn).

Application times: Twice daily, *viz* at 0, 8, 24, 32, 48, 56, 72, 80 and 192 h.

Reading times: As above and at 96, 198 and 216 h.

Experiment 3

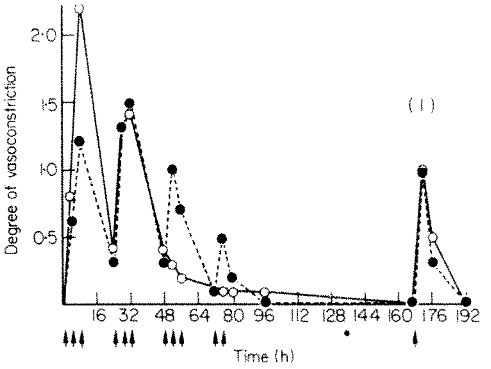
Steroid investigated: DMAC alone and 0·1 and 0·5% triamcinolone acetonide in DMAC.

Application times: The vehicle alone 3 times daily at 0, 4, 8, 24, 28 and 32 h followed by the steroid at 48, 52, 56, 72, 76, 80, 96 and 100 h.

Reading times: As above and at 104 h.

Results

These are shown in Figs 1 and 2. The vertical co-ordinate represents the average grade of vasoconstriction observed in the application sites from all the subjects for each steroid preparation investigated. The horizontal co-ordinate represents time (h) and the application times of the corticosteroids are



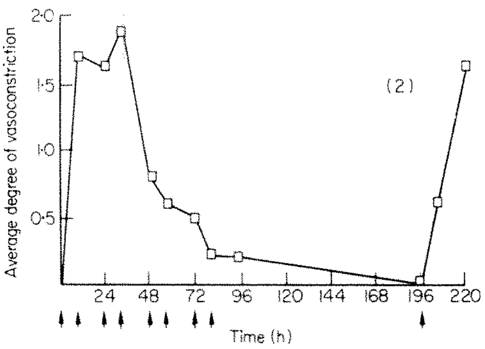


FIGURE 1. The vasoconstriction response diminishes rapidly with successive applications of a steroid (tachyphylaxis) but may be induced again after a rest period. ● – – – ● 0·1% triamcinolone in DMAC; ○——○ 0·5% triamcinolone in DMAC; arrow, time of application.

FIGURE 2. Tachyphylaxis to the blanching effect of topical fluocinonide cream. The blanching may be induced again after a rest period. □——□ 0·01 ml 0·05% fluocinonide cream; arrow, time of application.

indicated. It can be seen that the vasoconstriction responses diminish with succeeding applications (tachyphylaxis) but after a rest period the response returns. In Fig. 1 tachyphylaxis occurs more quickly to 0·5% than to 0·1% triamcinolone, suggesting that the stronger the corticosteroid the faster the onset of tachyphylaxis. Experiments using the vehicles alone for 48 h prior to the application of the steroids show that they neither caused vasoconstriction nor were responsible for the tachyphylaxis. Also once recovery from tachyphylaxis has occurred, it may be induced again if the steroids are applied repeatedly once more (du Vivier & Stoughton, 1975a).

ANIMAL STUDIES

A new technique of isolating radiolabelled epidermal DNA was used as previously described (du Vivier *et al.*, 1976) to determine the effect of increasing numbers of applications of steroid on epidermal DNA synthesis. Some mitotic counts were also performed. The technique involves hydroxyapatite, a compound which has a unique property of high affinity for DNA but none for RNA and protein. The latter may be washed through an hydroxyapatite column with a low phosphate buffer and then the DNA is ready for release from the hydroxyapatite with a high phosphate buffer. The amount of DNA present is calculated from the optical density recording on a spectrometer and the radioactivity of the material is measured in a scintillation counter so that the specific activity of the DNA (expressed as cpm per 10 μg DNA) may be determined. The advantage of the technique is its rapidity, accuracy and simplicity.

Whilst designing a model for screening for new therapies for psoriasis, using proliferating epidermal hairless mouse skin, we were surprised to find that five applications of a corticosteroid over a 54-h period was not nearly as effective an inhibitor of DNA synthesis as had been our experience with a similar number of applications of topical nitrogen mustard (du Vivier & Stoughton, 1975b). Increasing the number of applications to nine during the period had even less effect. It then seemed possible, in view of our findings with vasoconstriction, that tachyphylaxis might be occurring. Only one application of steroid was therefore made and profound inhibition of DNA synthesis was shown to occur. Because the effect of ultraviolet light used to stimulate epidermal proliferation in the model is short-lived, normal hairless mouse epidermis was investigated for the effect of repeated administration of steroids on DNA synthesis and mitosis.

Materials and methods

Animals. Random bred hairless mice (strain HRS/J) of either sex age 3–6 months.

Drugs. 0·05% fluocinonide cream (Metosyn) and propylene glycol (control).

Dose. 0·03 ml for each application.

Application site. The anterior aspect of the dorsum of the animals (henceforward called the neck). The posterior aspect (henceforth called the back) was left untreated to monitor for a possible systemic effect of the agent applied to the neck.

Tritiated thymidine. 25 μc (Amersham Searle, specific activity 15·7 Ci/mmol) was injected subcutaneously into the right thigh 1 h before sacrifice.

Sacrifice. At 3 p.m. the animals were sacrificed by ether inhalation. All procedures were performed at the same time of day to avoid diurnal variations in epidermal cell proliferation.

Proliferation epidermis

Hairless mouse skin was irradiated for 6 min at 20 cm using a germicidal lamp (General Electric G8T5) which causes increased mitosis, DNA synthesis and epidermal hyperplasia within 48–72 h (du Vivier & Stoughton, 1975b). The animals were sacrificed at 54 h after irradiation.

Application times for each experiment

Experiment 1. 48 h after irradiation.

Experiment 2. 1, 7, 24, 31 and 48 h after irradiation.

Experiment 3. 1, 3, 6, 9, 24, 27, 30, 33 and 48 h after irradiation.

Experiment 4. Because of the limited time span of the model (the ultraviolet light-induced burst of increased mitosis and DNA synthesis is short lived) applications were made twice daily for 2 days prior to irradiation and then on five occasions after irradiation thus, 48, 41, 24 and 17 h prior to irradiation and 1, 7, 24, 31 and 48 h after irradiation.

Number of animals used

Experiment 1. 12 (8 treated, 4 controls).

Experiment 2. 24 (12 treated, 12 controls).

Experiment 3. 24 (12 treated, 12 controls).

Experiment 4. 12 (8 treated, 4 controls).

Normal hairless mouse epidermis

The same drugs were used as above and were applied twice daily (9 a.m. and 4 p.m.) except on the day of sacrifice when they were applied at 9 a.m. only. There were seven experiments to demonstrate tachyphylaxis consisting of one, three, five, seven, nine, eleven and thirteen applications. Twelve animals (six treated, six controls) were used for each experiment. The length of action of one application alone of steroid was examined. Six treated animals and three controls were sacrificed 30h after one application and the same number were sacrificed 54 h after the application.

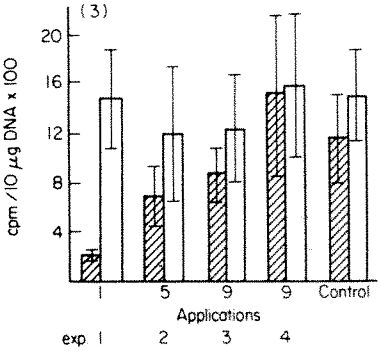
Analysis of results

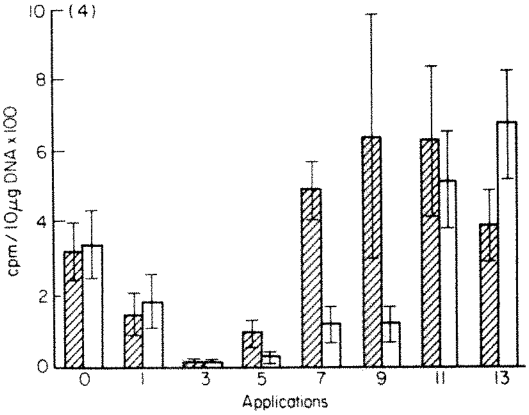
DNA synthesis was assessed for all tissues in all experiments using hydroxyapatite column chromatography. In normal epidermis the effect of three applications and of nine applications of topical corticosteroids on the mitotic index (expressed as number of mitoses per 1000 interfollicular basal cells) were also examined. Student's '*t*'-test was used for statistical evaluation of the results.

TABLE 1. Effect of increasing applications of steroids on mitotic index in normal epidermis

No. of applications	Steroid treated		Controls	
	Neck	Back	Neck	Back
3	5±2	4±2	20±8	15±9
9	26±11	10±7	15±6	20±4

Neck was treated. Back was left untreated to monitor for systemic effect.





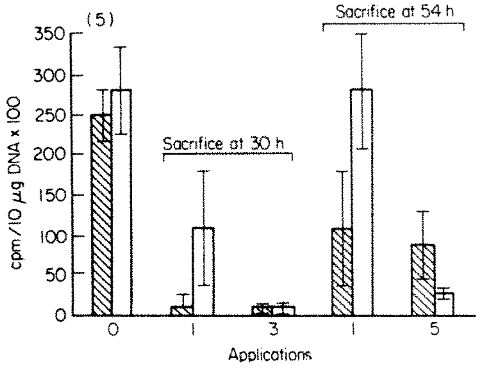


FIGURE 3. In proliferating epidermis one application of a steroid caused inhibition of DNA synthesis ($P < 0{\cdot}01$), five applications caused inhibition ($P < 0{\cdot}05$) but nine applications either over a 54-h period (Experiment 3) or over 5 days (Experiment 4) caused no significant inhibition of DNA synthesis. Hatched column, neck; open column, back; vertical rule, ±s.d.

FIGURE 4. Effect of increasing applications of steroids on DNA synthesis in normal epidermis (tachyphylaxis).

FIGURE 5. A steroid applied to the neck once only has a similar effect on DNA synthesis as three and five applications over 30 and 54 h respectively, but the systemic effect is less.

RESULTS

Proliferating epidermis. Fig. 3 shows an almost 5-fold depression of DNA synthesis ($P<0{\cdot}01$) after one application of steroid to the neck 6 h before sacrifice (Experiment 1). That the effect is local is shown by the lack of effect on the back skin of the animals. If the steroid is applied 5 times during a 54-h period (Experiment 2) the inhibition of DNA synthesis is only significant at the 5% level. If it is applied nine times either over a 54-h period (Experiment 3) or over 5 days (Experiment 4) there is no significant inhibition of DNA synthesis in comparison with controls.

Normal epidermis. Fig. 4 illustrates tachyphylaxis to the applied glucocorticoid. One application caused inhibition of DNA synthesis in the neck in comparison with controls ($P<0{\cdot}01$) and also in the back ($P<0{\cdot}05$) suggesting a systemic effect from the steroid. This trend continues, for after three applications over a 30-h period there is complete inhibition of the incorporation of tritiated thymidine into DNA in both the treated neck and untreated back area. After this however, recovery begins to take place. With seven applications DNA synthesis in the treated neck area is increased over controls ($P<0{\cdot}01$) and with thirteen applications DNA synthesis is the same as controls. Recovery is slower in the untreated back skin but after thirteen applications DNA synthesis is significantly increased over controls ($P<0{\cdot}01$). Mitotic counts were performed to confirm the results after three and nine applications of steroid (Table 1). After three applications there is profound inhibition of mitosis in both neck and back areas but complete recovery has taken place in the neck area, and partial in the back area, after nine applications so that acute tolerance to the cell inhibitory properties of steroids has occurred. The reason why a systemic effect should occur in normal but not in proliferating epidermis is unclear. Perhaps because the normal epidermis is thin, as opposed to the hyperplastic epidermis induced by irradiation, the steroid is better absorbed and therefore a systemic effect is seen in the untreated areas.

Fig. 5 shows that after 30 h one application of steroid inhibits DNA synthesis in the neck as profoundly as three applications and, after 54 h, one application is as effective as five applications during that time. However the systemic effect on the untreated back skin after one application is less at 30 h and non-existent at 54 h, unlike the effect of three and five applications.

DISCUSSION

Topical corticosteroids are the drugs most frequently used for the treatment of skin disorders yet there is no agreed schedule for prescribing these powerful agents. Whereas the administration of drugs systemically is fairly strictly controlled, topical therapy is less so. Even when individual physicians give precise instructions about the amount and frequency of applications, patients will vary considerably in adhering to these. In addition, side-effects of these drugs, both local (Epstein, Epstein & Epstein 1963, Sneddon, 1969), and systemic (Staughton & August 1975), are increasingly recognized and a concept of therapeutic scheduling is appropriate. Tachyphylaxis has been shown to occur with frequent application of steroids and this might suggest that less intensive therapy would be just as effective. Indeed after 30 and 54 h one application of a steroid had the same effect on DNA synthesis, as did three and five applications respectively. The only difference was that the greater number of applications caused marked inhibition of DNA synthesis in untreated sites (i.e. had a systemic effect) whereas this side-effect was much less with one application.

The mechanism of steroid-induced blanching of the skin is unknown but many workers have implied a relationship between catecholamines and glucocorticoids. Adrenaline and the α adrenergic agent noradrenaline injected intradermally cause vasoconstriction (Stoughton, DeOreo & Clendenning,

1960) whereas isoprenaline, a β-adrenergic agent, does not (personal observation) and Fritz & Levine (1951) showed that noradrenaline will not cause vasoconstriction in the meso appendix of adrenalectomized rats unless adrenal cortical extract is applied topically. With regard to mitosis, Bullough (1965) has shown that adrenaline is a powerful mitotic inhibitor in the presence of chalone, and hydrocortisone will potentiate this effect. It is therefore not surprising that tachyphylaxis, which is well known to occur with other drugs which act indirectly via catecholamines, should be demonstrated with topical steroids.

ACKNOWLEDGMENT

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DISCUSSION

Dr E.S.Snell (London): Have you repeated any of these vasoconstriction tachyphylaxis tests on diseased skin?

Dr du Vivier: No.

Dr C.J.Guerrier (Northampton): When you get down to zero vasoconstriction, if you change your steroid, do you once again achieve vasoconstriction?

Dr du Vivier: I do not think so. We think it is an effect of all steroids, so that changing the steroid, although it seems a good idea, does not make any difference.

Dr D.Boxley (Stoke on Trent): The repeated application of steroids to a condition such as rosacea appears to make it steadily worse, presumably due to vasodilatation. Did you see vasodilatation after repeated applications?

Dr du Vivier: We did not observe that in this group of experiments. However Dr Stoughton has occasionally seen a subject who gets erythema as opposed to vasoconstriction.

Professor M.W.Greaves (London): It is fashionable to give prednisolone maintenance treatment on alternate days, and it may be that something like that sort of regime ought to be tried in the use of topical steroids. Have you tried it?

Dr du Vivier: No. Considered it, yes, but not tried it.

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The clinical assessment of topical corticosteroid activity

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SUMMARY

Glucocorticoid-induced vasoconstriction of human skin has provided a useful guide to topical anti-inflammatory activity. The concentration and base in which a steroid is used influence this activity, and traditional greasy ointments are probably the most effective vehicles. Confirmation of clinical effectiveness should be by double-blind comparison in patients with bilateral skin lesions. Psoriasis provides a more discriminating test of steroidal activity than eczema; the use of occlusive dressings may mask differences between compounds. Short-term trials are informative but in patients with chronic disease, longer studies should also be done.

The likely clinical usefulness of a topically active corticosteroid must be judged in the light of the systemic effects likely to arise from its absorption through the skin, and its propensity to cause undesirable local skin changes, as well as its topical anti-inflammatory activity. The first two unwanted effects are discussed elsewhere in this volume and it is solely with the assessment of clinical activity that this paper is concerned.

For a decade screening tests of steroids for topical anti-inflammatory activity were based on the assessment of a compound's potential for preventing or ameliorating artificially induced inflammation in small laboratory animals. Although such tests are still used by some workers and may have a confirmatory role to play they have shortcomings: there is generally a species difference in the response, which makes interpretation of the results difficult; reproducibility is not good; and the tests tend to be too time-consuming for screening large numbers of compounds.

It has been suggested that a modified cotton pellet granuloma test predicts topical anti-inflammatory activity. A cotton pellet impregnated with steroid is implanted subcutaneously in the rat; the difference in the reduction in weight of granulomatous tissue, compared with that surrounding a similar but non-impregnated cotton pellet implant, being held to be a measure of topical activity. However, although this test has the attraction of enabling the systemic activity of a compound to be assessed simultaneously (by measuring any reduction in thymus gland weight), as an indication of topical anti-inflammatory potential it must be viewed with reservation. Surgical introduction, rather than penetration of the intact epidermal barrier, makes it doubtful if a corticosteroid with activity in this model is exerting a true topical, as distinct from a localized effect. Although it can be argued that in inflammatory skin disease the barrier to absorption is often disrupted, in some conditions—

characterized by intense lichenification, or on certain sites, such as the palms and soles—ability to penetrate keratin must be the prerequisite for clinical topical activity. Reliance on activity in the cotton pellet granuloma test—which may be a reflection of low diffusability or of resistance to local metabolic degradation—could result in the selection of a glucocorticoid which does not satisfy this primary requisite.

The vasoconstriction assay for topical anti-inflammatory activity (McKenzie & Stoughton, 1962) lacks the principal disadvantages of previous screening tests. It is performed in human skin and thus avoids the problem of interpreting the relevance of animal data; secondly, the demonstration of the blanching response must be partly a measure of a compound's ability to penetrate intact human stratum corneum; finally, the assay is a practicable method of screening a large number of compounds. Although steroid-induced vasoconstriction may vary according to the site of application on the forearm, between arms in the same subject, and between subjects (all of which variables may be in part compensated for by good experimental design), the test has been a useful guide to the selection of glucocorticoids with clinical topical activity.

Predictions of topical anti-inflammatory activity by the vasoconstriction test require clinical confirmation, and a long personal experience of this process suggests that some important principles are involved. Where vasoconstriction values are quoted these are figures from our own laboratories, using ethanolic solutions of pure substances (McKenzie & Atkinson, 1964); other workers have used differing methods and times of application of test compounds, or used formulated substances, and their results are thus not necessarily comparable.

Clinical trial

The vasoconstrictor activity of five corticosteroids is compared with that of fluocinolone acetonide, assigned an arbitrary value of 100, in Table 1. While triamcinolone acetonide has about three-quarters, and flurandrenolone about half the activity of the standard, it will be seen that both hydrocortisone and betamethasone alcohol exhibit less than 1%. By contrast, betamethasone valerate has greater than three times the activity of fluocinolone acetonide.

TABLE 1. The vasoconstriction values for the six topical steroids compared clinically by Williams *et al.* (1964)

Fluocinolone acetonide	100
Hydrocortisone	< 1
Flurandrenolone	46
Triamcinolone acetonide	75
Betamethasone	< 1
Betamethasone valerate	360

An 0·1% ointment of betamethasone valerate was compared with the standard marketed ointments of each of the other five steroids by Williams *et al.* (1964). The trials were double-blind, within patient studies and each comparison was analysed sequentially in four groups depending upon the disease treated—eczema or psoriasis—and whether or not occlusive dressings were used.

Sequential analysis (Armitage, 1960), which is simple and economical of patients, is a useful method of evaluating the results of clinical comparisons of steroids. The clinician, having entered a patient into the trial because he had bilateral disease of equal severity, asks himself when assessing the effect of treatment, 'Is there a difference between these lesions when previously there was none?' He then records a choice ('preference') or, if there is no difference, an equal response ('tied pair'). As each patient completes the trial his record card is passed to an independent observer who decodes the result and plots it sequentially. When the advancing line which represents the number of preferences expressed bisects one of the pre-set boundaries (usually corresponding with the 5% level of significance) no more patients need be entered in the trial. Usually provision is made on the record card for the patient, as well as the physician, to express a preference for one or other treatment. Patient preferences closely correspond with those of the physician and give little added therapeutic information; however they are valuable in assessing such things as irritancy or stinging. There is no evidence that the use of more elaborate types of analyses, such as the assigning of a numerical value to the degree of scaling or infiltration, provides more information.

In none of the twenty groups in Williams' trial was betamethasone valerate less effective than the steroid with which it was compared and in fourteen of the twenty the differences were statistically significant. In one of the comparisons of betamethasone valerate with flurandrenolone, that in which patients with eczema used occlusive dressings, there was no difference in response. There were signs of this tendency for flurandrenolone to perform clinically rather better than one might predict from its vasoconstrictor value in the other groups also (Fig. 1). It looks as if the screening test somewhat overrates triamcinolone acetonide and under-rates flurandrenolone. This point is again borne out in the overall percentage of patients showing an equal response to treatment with betamethasone valerate (Table 2). In this type of trial the number of equal responses, relative to the number of preferences, tends to be larger as the difference between the clinical potency of the two treatments diminishes. As will be seen there is little difference between the figures for triamcinolone acetonide and flurandrenolone. However, with this exception, the clinical topical activity of the group accords with the activity predicted by the vasoconstriction test. All but betamethasone (which is now no longer marketed) have been in use for more than 10 years and experience has confirmed this ranking order of clinical effectiveness.

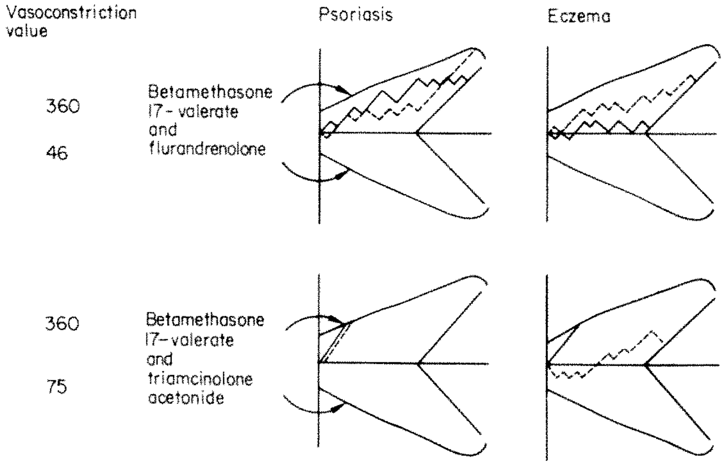

FIGURE 1. A comparison of the vasoconstriction value, and the clinical activity, of flurandrenolone and triamcinolone acetonide with that of betamethasone valerate. (Adapted from Williams *et al.*, 1964.) —— occluded; - - - - non-occluded.

Table 3 gives the vasoconstriction values for six corticosteroids and includes the later compounds, clobetasol propionate, clobetasone butyrate and fluclorolone acetonide. Clobetasol propionate has

TABLE 2. The percentage of patients with eczema or psoriasis responding equally to betamethasone valerate and five other topical steroid ointments, compared with the ranking order of the steroids in the vasoconstrictor test

	Equal clinical responses (%)	Vasoconstriction ranking order
Fluocinolone acetonide	50	1
Flurandrenolone	46	3
Triamcinolone acetonide	42	2
Betamethasone phosphate	36	4
Hydrocortisone	24	5

(Adapted from Williams *et al.*, 1964.)

been shown to have greater clinical activity than betamethasone valerate and fluclorolone acetonide (Sparkes & Wilson, 1974; Björnberg & Hellgren, 1975; Corbett, 1976) and clobetasone butyrate to have greater clinical activity than either hydrocortisone (Munro & Wilson, 1975) or flurandrenolone. Clobetasol propionate has been in general use for more than 2 years and it is my impression that the results of the early clinical trials, if anything, underestimated its high topical anti-inflammatory activity. Clobetasone butyrate has not been widely available for long enough to justify further comment.

TABLE 3. The vasoconstriction values for six topical steroids compared with fluocinolone acetonide given an arbitrary value of 100

Fluocinolone acetonide	100
Hydrocortisone	< 1
Flurandrenolone	46
Clobetasone butyrate	263
Betamethasone valerate	360
Fluclorolone acetonide	413
Clobetasol propionate	1869

The vasoconstriction test employs simple ethanolic solutions whereas clinical confirmation requires the use of ointments or creams; as comparisons often involve the use of commercially available formulations, the concentration of the steroids tested and the composition of their bases may differ. However, as with the first group of compounds discussed, in general, clinical activity seems to be broadly in agreement with activity in the blanching test.

The effect of concentration

One suspects that with the early synthetic glucocorticoids, the selection of a concentration for marketing depended largely on guess work. More recently attempts have been made to titrate the concentration in the clinic. Although factors other than simple concentration of the active ingredient are extremely important (and are discussed below), there is no doubt that there is a dose (concentration) response relationship. This relationship may not be a straight line and there is circumstantial evidence of a plateauing effect. Nowadays, with compounds of high innate activity, the goal is not maximal anti-inflammatory effect but some selected point between that and an acceptable (and minimal) risk of side-effects; for this, preliminary dose–response trials are mandatory.

The results of such a study are shown in Fig. 2. Clobetasone butyrate, in increasing concentrations 0·01, 0·025 and 0·05%, was compared with 1% hydrocortisone in patients with eczema. At the lowest concentration clobetasone butyrate and hydrocortisone were equally effective; at 0·05% clobetasone butyrate was significantly more effective than 1% hydrocortisone and used in the intermediate concentration it gave intermediate results. In a stringent test of the effect of percutaneously absorbed clobetasone butyrate on hypothalamic pituitary adrenal (HPA) function there was less effect using an 0·05% than an 0·1% ointment (Munro & Wilson, 1975) and in an animal model of epidermal atrophy a similar concentration effect was noted (Winter, personal communication). Thus, for this steroid, 0·05% was the optimum concentration.

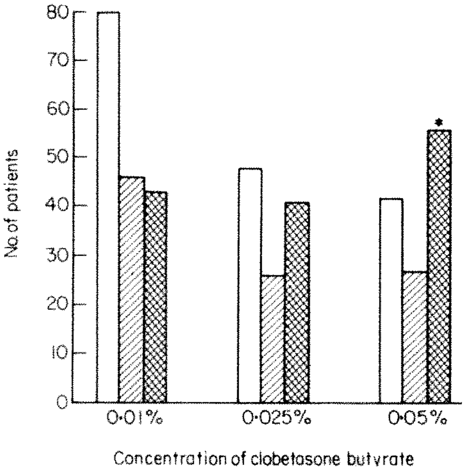

FIGURE 2. The comparative response of patients with bilateral eczema to hydrocortisone 1% and clobetasone butyrate creams. (From Munro & Wilson, 1975.) Open column, equal response; hatched column, better response to 1% hydrocortisone; cross hatched column, better response to clobetasone butyrate; * $P < 0{\cdot}05$.

In a double-blind study in patients with symmetrical butterfly lesions of discoid lupus erythematosus Warner & Hanson (personal communication) were unable to detect a difference in response to betamethasone valerate whether or not the concentration of steroid used was 0·1% or 1%. This suggests a flattening of the dose response curve, at least for this steroid, in this disease.

The effect of formulation

It is well recognized that the formulation in which a steroid is presented can influence its clinical efficacy. Modifications of the vasoconstrictor test have been used to assess the release of a steroid from its base (Busse *et al.*, 1969; Barry & Woodford, 1974; Burdick, 1974). As a measure of bio-availability this test is useful. However, in our experience base-dependent differences in the intensity and duration of blanching noted in the laboratory may not necessarily reflect differences in anti-inflammatory activity of sufficient magnitude to be detectable clinically.

The addition of penetrants, such as propylene glycol, DMSO and urea, can facilitate the absorption of a steroid. They may be used either to increase the anti-inflammatory activity of a formulation by carrying more steroid through the skin barrier, or to enable a given anti-inflammatory effect to be achieved with a lower concentration. If the unwanted effects of topical steroids are dependent on the amount absorbed it might be argued that neither of these goals is worth pursuing. For example, if the use of penetrants lifted the clinical activity of hydrocortisone to the level of potent topical preparations, in addition to potential HPA suppression sodium retention might become a problem with this steroid which has mineralo–corticoid properties. On the other hand, halving the concentration of a potent topical steroid, yet retaining the same clinical activity by doubling absorption with a penetrant, would seem to give no benefit to the patient as presumably the potential for side-effects would remain the same.

A more important property of bases may be occlusivity. Baker (1969) has shown that the application of white soft paraffin profoundly reduces transepidermal water loss, much more so than do hydrophilic preparations. This may explain the results of Munro *et al.* (1976) who compared betamethasone valerate as an ointment with fluocinonide in FAPG base in patients with eczema or with psoriasis, and in both diseases found betamethasone valerate to be significantly more effective. This was contrary to the findings of Rosenberg (1971), Meenan (1972) and Portnoy & Sarkany (1972) in similar trials in which betamethasone valerate was used as a cream; these indicated either that fluocinonide in FAPG was more effective than betamethasone valerate cream or that there was little difference between them.

It may be that any advantage of formulating a glucocorticoid in a base, which combines some of the properties of an ointment with some of those of a cream, is offset by a loss of activity as compared with traditional greasy ointments.

The effect of the choice of disease

Patients' responses to topical steroid treatment may differ. This is not surprising, especially as steroids are used to treat a variety of very different diseases. The response to a given glucocorticoid of eczema can be markedly different to that of psoriasis. When comparing two reasonably potent steroids a difference between their performance is more likely to be apparent if psoriasis rather than eczema is the disease studied. In Fig. 3 the response of eczema and psoriasis to betamethasone valerate compared with fluocinolone acetonide, and to fluclorolone acetonide compared with clobetasol propionate, is

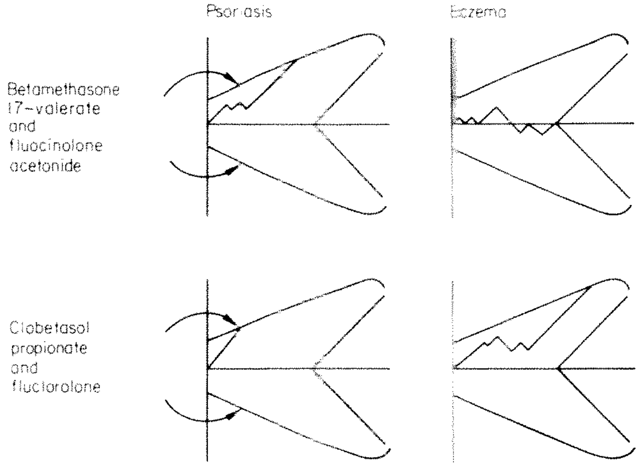
Psoriasis
Eczema
Betamethasone 17-valerate and fluocinolone acetonide
Clobetasol propionate and fluclorolone

FIGURE 3. Two examples from clinical trials in which psoriasis is shown to be a more discriminating test of steroidal activity than eczema. (Top figures adapted from Williams *et al.*, 1964; bottom figures adapted from Sparkes & Wilson, 1974.)

seen. Whereas fewer patients with psoriasis have to be observed (almost all the preferences being for betamethasone valerate and clobetasol propionate respectively) a larger number is needed to illustrate the differences between the pairs of steroids when treating eczema—fewer of the preferences being for one treatment. This is reflected in the numbers of patients who showed an equal response, there being fewer in the psoriasis than in the eczema group. In Table 4 the percentage of equal responses in the Williams *et al.* (1964) trial are broken down by disease. The inverse correlation with potency shown in Table 2 persists for each separate disease but it becomes very obvious, particularly in respect of triamcinolone acetonide and betamethasone, that psoriasis provides a topical steroid with a more discriminating test than eczema.

TABLE 4. Percentage of patients showing an equal response to betamethasone valerate ointment compared with five other topical steroid ointments in patients with eczema and psoriasis

Corticosteroid	Eczema	Psoriasis
Fluocinolone acetonide	56	43
Flurandrenolone	50	44
Triamcinolone acetonide	51	27
Betamethasone	43	18
Hydrocortisone	27	18

(Adapted from Williams *et al.*, 1964.)

It is possible that some types of eczema, for instance lichen simplex or discoid eczema, would provide as sensitive a model as psoriasis for testing steroids. However their comparative rarity, and the difficulties of standardization of diagnosis if results from various centres are combined, make this impracticable. The heterogeneous group which for clinical trial purposes we refer to as eczema probably contains a high proportion of patients with relatively minor skin disease who respond readily and equally to a topical steroid with either moderate or high activity.

The effect of occlusive dressings

The use of polythene occlusive dressings enhances percutaneous absorption and thus the clinical efficacy of topical steroids. This enhancement can blur differences between the response to steroids under trial. The effect is especially noticeable in eczema, and is illustrated in Fig. 4. Munro, Wilson &

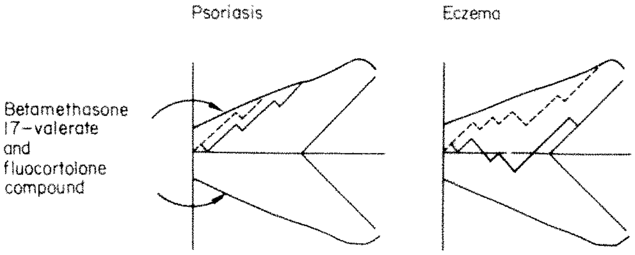

FIGURE 4. The use of occlusive dressings in patients with eczema, masking the difference between two topical steroids. (From Munro *et al.*, 1967.) —— occluded; - - - - non-occluded.

Rhodes (1967) showed the response of eczema and psoriasis to betamethasone valerate to be superior to that to fluocortolone except where occlusive dressings were used in patients with eczema. In this group there was no significant difference between the two steroids. However, in patients with psoriasis the use of occlusion was not sufficient to lift the activity of fluocortolone to that of betamethasone valerate in this less responsive disease.

The effect of length of treatment

The standard, within patient, comparison of two topically active steroids which has been employed in all the examples cited has traditionally been of short duration—of the order of 7–21 days. Many patients' lesions will respond to active preparations very quickly and the first review of out-patients should not be delayed beyond 1 week.

Sequential analysis can be viewed as an attempt to disprove the null hypothesis—that there is no difference between the preparations A and B. If by using a short term of treatment, say 7 days, the hypothesis is refuted, then statistically speaking the requirements of the trial have been met. I believe the clinical requirements have been satisfied also in that I know of no evidence that if steroid A is more effective than steroid B in the short term (whether or not complete healing of the skin has been achieved) on continuing treatment steroid B will prove to be the more effective of the two. It is quite widely believed that the longer a trial continues the less chance there is of showing differences between preparations. This seems logical in that patients with, for instance, relatively trivial eczema, treated under trial conditions with two reasonably effective steroids and reviewed for the first time after 4 weeks, may well show complete bilateral healing. The fact that one side was healed after 7 days and the other only after 21 days will be obscured and the erroneous conclusion arrived at that, in this patient, the response to the two steroids was equal.

However, in treating patients with chronic or the less responsive diseases, short-term trials, although of undoubted value, may not reveal the full therapeutic potential of a steroid. A clinical impression, spontaneously reported from various workers, that patients with psoriasis tended to remain free of disease for longer after treatment with clobetasol propionate than other topical steroids lead Floden *et al.* (1975) to undertake a longer-term study. They carried out a within-subject comparison of clobetasol propionate with fluocinolone acetonide for 6 months in twenty-nine patients with psoriasis. Their results not only confirmed their earlier impressions of the longer remissions induced on the clobetasol propionate-treated sides but, interestingly, showed that the response to clobetasol propionate increased with time: at one week 64% of preferences were for clobetasol propionate and the percentage rose at 2 weeks and again at 3 weeks until at 4 weeks, and thereafter at monthly intervals throughout the trial, the choices for clobetasol propionate never fell below 90%. Corbett (1976) compared clobetasol propionate with betamethasone valerate, in a smaller group of patients, in a similarly designed study and found a similar trend.

CONCLUSION

The vasoconstrictor assay, using ethanolic solutions of glucocorticoids on intact human skin, provides a useful indication of clinical topical anti-inflammatory activity. There is no evidence that the fact that such clinical activity is associated with an ability to induce skin blanching is causal. Indeed, the recent observation by duVivier & Stoughton (1975) that there is rapidly occurring tachyphylaxis to steroid vasoconstriction, in the absence of evidence of diminishing clinical anti-inflammatory activity, indicates that an active glucocorticoid does not depend for its clinical effectiveness primarily on the ability to vasoconstrict—no matter how useful in treating inflamed skin this property might seem to be.

Vasoconstriction does not provide a precise measure of anti-inflammatory activity and an example has been given of an apparent under-rating (flurandrenolone) and over-rating (triamcinolone acetonide) of two compounds by this assay. Nevertheless, so far all compounds with a high vasoconstriction rating have performed well clinically in comparison with those with only a moderate rating and compounds such as hydrocortisone, with very low vasoconstrictor values, are easily distinguished clinically from high rating steroids.

The formulation of compounds is critical in that the clinical activity of a preparation is dependent upon the intrinsic activity of the glucocorticoid, the concentration used and the characteristics of the base in which it is formulated. There is good evidence that greasy ointment bases are more effective vehicles than are creams or bases which attempt to bridge the two.

Differences between steroids are more likely to be apparent if psoriasis rather than eczema is used as the test disease, and the use of occlusive dressings is avoided. There seems to be no justification for comparing different preparations in separate groups of patients, indeed in the patient with bilateral disease the clinical research worker is fortunate in having the perfect accessible, matched control. There is now evidence that the full investigation of new steroidal compounds should include long- as well as short-term trials.

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DISCUSSION

DR R.C.D.STAUGHTON (LONDON): We know that a number of steroids, especially strong ones, have a systemic effect. What would be your comment on the changing of a rash in a symmetrical distribution as regards the systemic effect of the cream put on one side compared with the cream put on the other side?

MISS LYN WILSON: It has been shown several times that when you compare an active steroid with a placebo in a double-blind trial, the steroid side gets better and the placebo side does not. These compounds are working topically and not systemically.

British Journal of Dermatology (1976) **94**, Supplement **12**, 43.

The treatment of vitiligo with topical corticosteroids

LIGHT AND ELECTRONMICROSCOPIC STUDIES

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SUMMARY

Twenty patients with lesions of vitiligo were treated either with 0·1% betamethasone valerate (BV) or with 0·05% clobetasol propionate (CP) creams and similar control areas with placebo preparations.

After 3 months of treatment three of the ten patients treated with BV showed a significant amount of repigmentation only in the steroid-treated areas. Only one of the ten patients treated with CP showed partial repigmentation.

Using both light and electron microscopy, histological studies of biopsies taken from control- and steroid-treated areas showed a marked repopulation with functional melanocytes in the repigmenting vitiliginous skin. The melanocytes in these steroid-treated repigmented areas were more dendritic and dopa-positive and, unlike melanocytes in the pigmented margins of untreated areas of vitiligo, contained many melanosomes of normal size, shape and melanization.

It appears that both topical corticosteroids are of use for the treatment of selected patients with vitiligo and can induce repigmentation of the skin.

In recent years, several studies have indicated that corticosteroid preparations can promote the repigmentation of vitiliginous skin (Kandil, 1970; Koopmans-Van Dorp *et al.*, 1973; Kandil, 1974). This paper reports the results of treatment with either 0·1% betamethasone valerate (Betnovate) (BV) or 0·05% clobetasol propionate (Dermovate) (CP) creams on twenty patients with vitiligo. These topical preparations were applied to selected areas of vitiligo and compared with similar control areas treated with placebo creams. Four patients showed significant repigmentation of the steroid-treated vitiliginous areas of skin.

PATIENTS AND METHODS

Twenty patients with symmetrical areas of vitiligo were selected for treatment (Table 1). The age range of the patients was 14–66 years (mean 34 years); 15 were female and 5 male. Only three of the patients were coloured and of various racial origins, the remainder were British and white. The steroid-treated and control areas of vitiligo were photographed before the start and at various times during treatment, both in black and white and in colour. These serial photographs were used to assess the response to treatment and the amount of repigmentation of the skin. The steroid and placebo creams were applied

TABLE 1. Patients with vitiligo treated with Betnovate (BV) or Dermovate (CP)

Case no.	Sex and age	Racial colour	Sites treated	Length of treatment (months)	Steroid used	Response % repigmentation
1	F 46	White	Hand & trunk	4	BV	0
2*	F 15	White	Knees & elbows	12	BV	90
3*	F 23	White	Hands	6	BV	0
4*	M 66	White	Face & hands	6	BV	30
5*	M 22	Coloured	Arm	5	CP	< 10
6*	F 26	White	Arms	6	CP	20
7	F 25	White	Knees, elbows & hands	5	CP	10
8	F 14	White	Knees & feet	5	CP	< 10
9	F 42	White	Face & hand	4	BV	0
10	F 62	White	Hand	4	BV	0
11	F 58	White	Hand	4	CP	0
12*	F 18	White	Back & wrist	12	BV	60
13	F 49	White	Hand	4	CP	0
14	F 25	White	Hand	4	BV	0
15	F 30	White	Back	4	CP	0
16	M 34	Coloured	Hand	4	CP	0
17	M 37	White	Arm & hand	4	CP	< 10
18	F 29	Coloured	Back & hand	4	BV	< 10
19	M 32	White	Arm	4	BV	0
20	F 33	White	Hands	6	CP	0

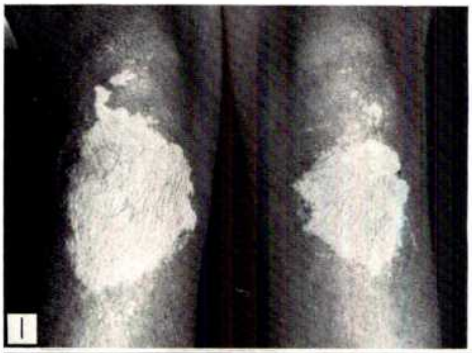
* Patients biopsied.

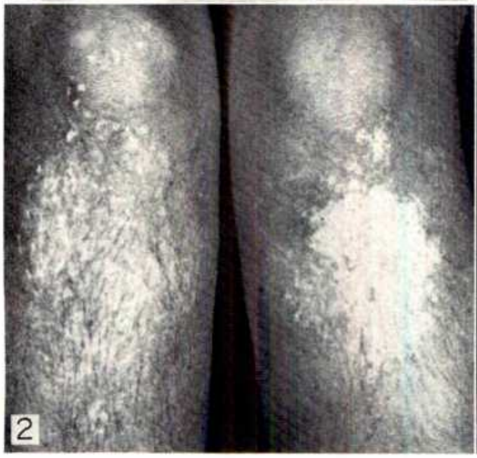
twice daily, the patient being instructed to apply either BV or CP to one side and a placebo cream to the other side. The patients were seen at monthly intervals and after 4–5 months treatment was either abandoned or the steroid cream was then applied to both sides.

Shave biopsies of skin were taken from steroid- and placebo-treated areas of vitiligo, as well as from adjacent areas of pigmented normal skin, in six of the patients after 4 months of therapy. Parts of the specimens were split in 2N NaBr solution at 37°C for $1\frac{1}{2}$ h (Staricco & Pinkus, 1957) and the epidermal sheets were then incubated in 1 : 1000 L-Dopa solution at 37°C for 4 h. A portion of each biopsy specimen was embedded in paraffin after fixation in 10% formal saline and vertical sections cut and stained with haematoxylin and eosin and a silver stain for melanin. Portions were also fixed in 3% gluteraldehyde. These were embedded in araldite and ultra-thin sections were cut and stained and then viewed in the electron microscope.

RESULTS

The results of treatment are shown in Table 1. Three of the ten patients treated with BV showed marked repigmentation of the steroid-treated areas as did one patient treated with CP. Repigmentation was first noticed after 2 months of treatment. No significant repigmentation occurred in control areas treated with placebo creams. The first sign of a response to treatment was the appearance of perifollicular islands of pigment around the normally pigmented hairs in the area of vitiligo (Figs 1–3). These islands of pigment gradually coalesced. In addition there was a gradual spread of pigment from the margins of the lesions into the amelanotic areas. In Case 2, the repigmentation of the skin was





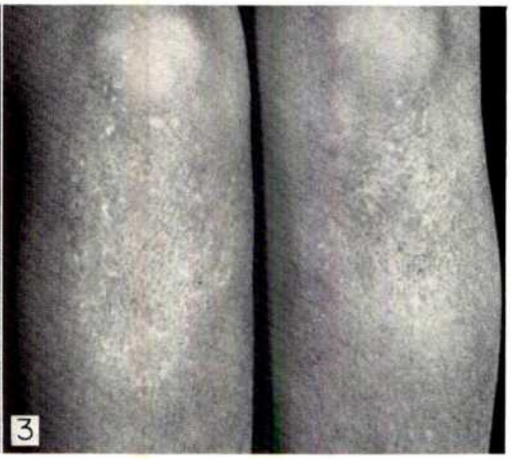


FIGURE 1. Case 2, areas of vitiligo on knees.

FIGURE 2. Case 2, right knee treated for 4 months with Betnovate (BV) and showing substantial repigmentation.

FIGURE 3. Case 2, both knees almost completely repigmented after 1 year of treatment (BV having been used for both after the first 4 months).

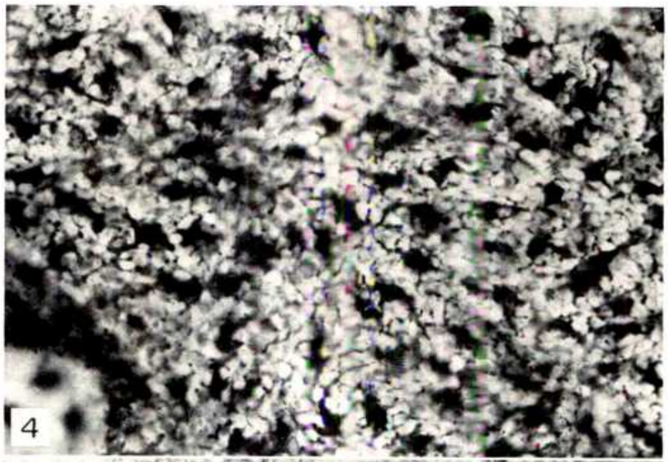
almost complete in the steroid-treated areas and there has been no loss of pigment in the 4 months since treatment has been discontinued. It appeared that the younger the patient the better the response to therapy; however, in the older patients the backs of the hands were treated, and vitiligo in these sites is more resistant to treatment. Areas of vitiliginous skin in which the hair was also depigmented did not respond.

As regards side-effects, it was observed that the majority of patients treated with either BV or CP had varying degrees of steroid-induced atrophy of the skin. One elderly patient after 4 months of treatment with BV developed striking ecchymoses on the back of the steroid-treated hand. One lady (Case 9) had to stop treatment to her face after 3 months because of a Betnovate induced rosacea that rapidly responded to systemic therapy with oxytetracycline. Only one patient (Case 16) considered that the steroid-treated area of vitiligo had got worse, though there was no objective evidence for this. Several patients also noted a few pustules in the steroid-treated areas.

Vertical sections of biopsies taken from steroid-treated repigmented areas showed pigment in the epidermis, but also atrophy of the skin when compared with sections from similar placebo-treated areas. The effects of 4 months of treatment with these topical steroids and placebo creams on the melanocyte population of vitiliginous skin and on normal pigmented skin are shown in Table 2. In

TABLE 2. Effect of 4 months' treatment with topical steroid or placebo cream on melanocyte populations of vitiliginous skin

Case no.	Topical steroid used	Site	Average no. of melanocytes/mm² ± s.e. mean		
			Pigmented normal	Steroid-treated	Placebo-treated
2	BV	Knee	1874 ± 49	829 ± 68	11 ± 5
3	BV	Hand	—	0	0
4	BV	Hand	1760 ± 59	426 ± 61	0
5	CP	Forearm	1517 ± 65	24 ± 11	0
6	CP	Arm	—	10 ± 4	0
12	BV	Back	1120 ± 45	758 ± 72	7 ± 3



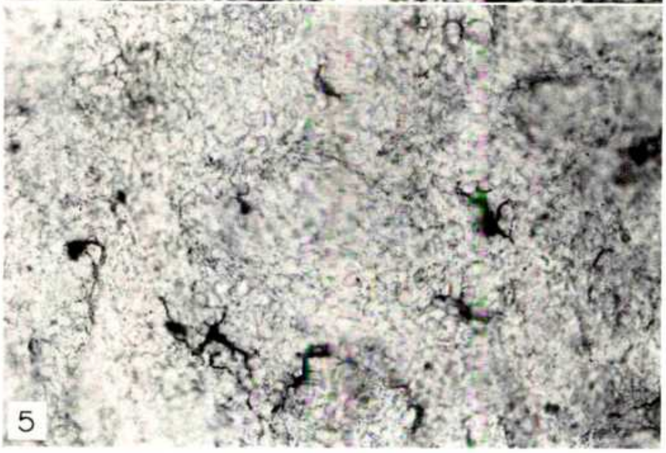


FIGURE 4. Dopa-incubated epidermal sheet taken from Betnovate-treated and repigmented area of skin showing many dendritic and dopa-positive melanocytes (× 320).

FIGURE 5. Dopa-incubated epidermal sheet from area treated with placebo cream showing only a few melanocytes (× 320).

biopsies from three of the patients there was a striking repopulation of the vitiliginous skin with dopa-positive melanocytes. The number of melanocytes per mm^2 in the repigmented steroid-treated skin was considerably more than in the control placebo-treated areas, though not as many as in the adjacent control pigmented area of skin. The melanocytes in the epidermal sheets from the steroid-treated repigmented areas appeared to be smaller than normal and had many dendrites and were strongly dopa-positive (Fig. 4). In contrast in the placebo-treated areas there were only a few melanocytes which were usually only weakly dopa-positive with only a few dendrites (Fig. 5). The repopulation of the epidermis with functional melanocytes appeared to start around hair follicles with the melanocytes spreading from the external hair root sheaths.

Ultrastructural studies of biopsies taken from amelanotic areas of vitiligo showed that in addition to a complete lack of melanosomes in the epidermis, there was a total absence of melanocytes. Langerhans cells were present and were recognized by their characteristic organelles. Biopsies taken from the margins of areas of vitiligo showed a lack of melanosomes in the keratinocytes of the epidermis (Fig. 6). A few melanocytes could be found and these mainly contained a few rounded melanosomes that were incompletely melanized. These melanocytes had very prominent microfilaments and micro-

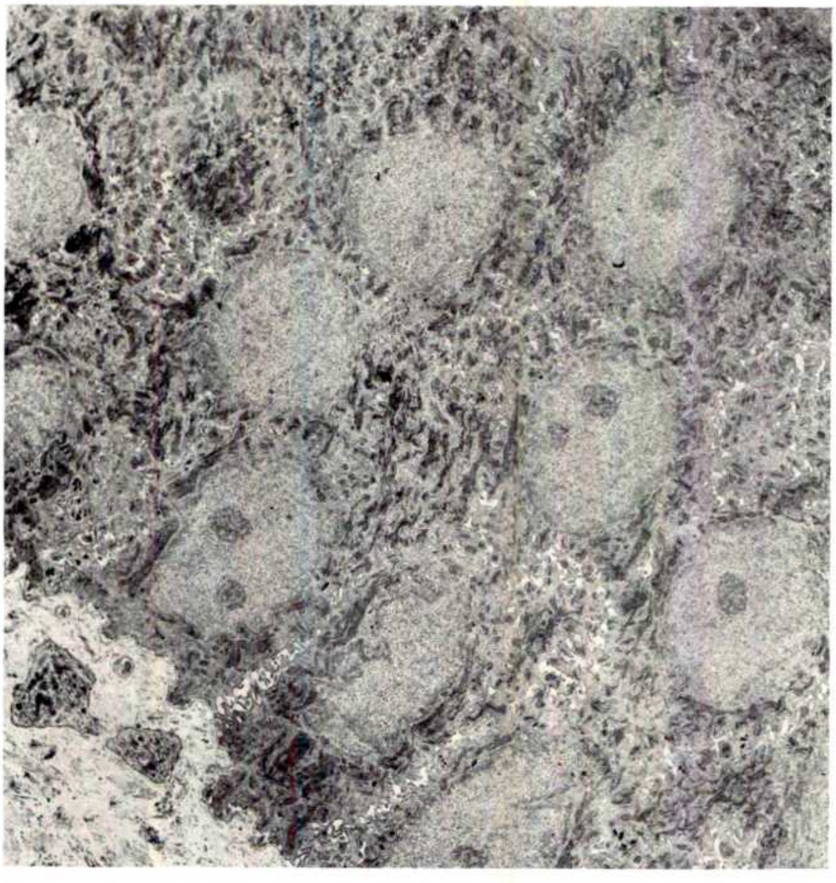


FIGURE 6. Electron micrograph of a biopsy from edge of area of vitiligo showing epidermal cells containing only a few melanized melanosomes (× 3000).

tubules. Several degenerating melanocytes were found in the margins of areas of vitiligo and in addition to vacuoles they contained swollen and disrupted mitochondria.

Electron microscopy of the steroid-treated and repigmented areas of vitiligo did not reveal any significant difference from the normal pigmented skin (Fig. 7). The melanocytes in the basal layer of the epidermis contained ellipsoidal melanosomes in various stages of melanization. Melanosomal complexes were present in the keratinocytes mainly in the basal portion of the epidermis and were often arranged in aggregates above the tops of the nuclei.

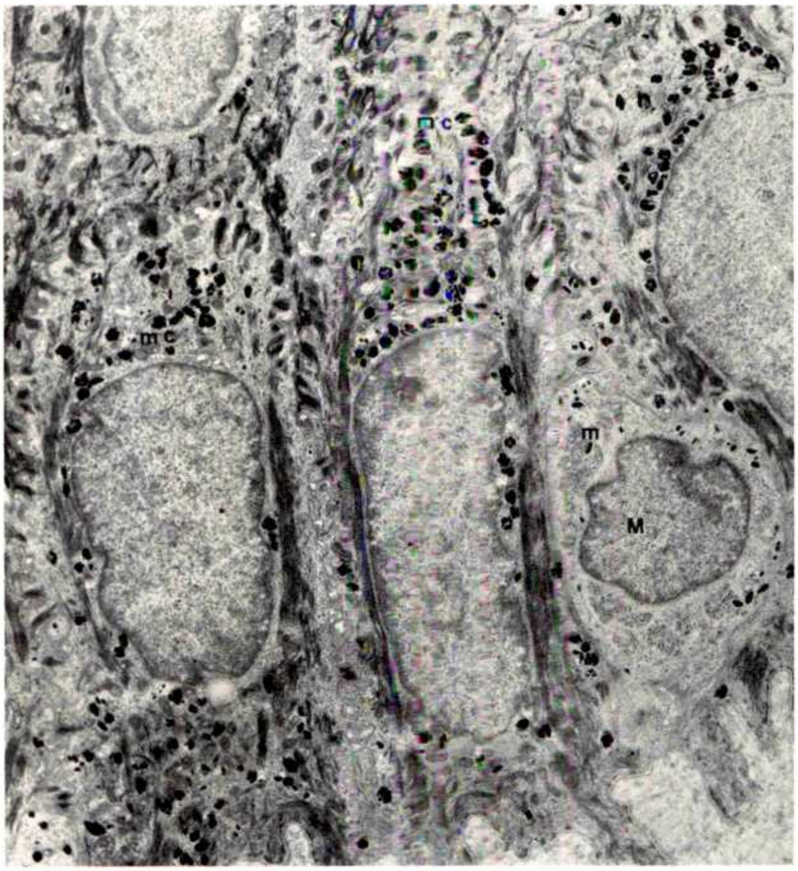


FIGURE 7. Electron micrograph of biopsy from a steroid-treated repigmented area of vitiligo showing many melanosomal complexes (mc) in keratinocytes and developing melanosomes (m) in a melanocyte (M) (× 5000).

It was noted that in both the placebo- and steroid-treated areas of vitiligo there was an increased number of dermal macrophages some of which contained phagocytosed melanin in lysosomal structures.

DISCUSSION

The treatment of vitiligo is unsatisfactory at present. Treatment with systemic psoralens combined with exposure to long-wave ultraviolet light, including natural sunlight, is only effective in about a quarter of the patients treated in the U.K. (Bleehen, 1972, 1975). Better results with psoralens have been obtained in countries where sunshine is readily available (Sehgal, 1973) and with higher doses of systemic psoralens (El Mofty, 1968; Ormea, 1974; Fitzpatrick, Parrish & Pathak, 1975). There is an appreciable percentage of patients who do not respond at all to treatment with psoralens and it is these who may be helped using topical corticosteroid preparations.

The results of this trial are somewhat disappointing and not as good as has been reported by Kandil (1974). However, these results are better than those of El Mofty & Nada (1974) who, using betamethasone valerate in collodion, found that none of their patients treated with topical steroid alone had any response to therapy. In this trial 20% of the patients had some response to protracted therapy. A more rigorous double-blind trial using clobetasol propionate lotion is in progress and will be reported.

The side-effects of these potent topical steroid preparations will limit their usefulness in the treatment of vitiligo. In many patients the vitiligo on their face is the main cosmetic disability and it would be unwise to treat this site for several months with topical steroids.

Histopathological studies on vitiliginous skin have shown that there is a lack of melanocytes in the epidermis (Jarrett & Szabo, 1956; Birbeck, Breathnach & Everall, 1961; Breathnach, 1975). How these melanocytes are lost is still a matter of speculation and various theories have been put forward as to the aetiology of vitiligo and how the melanocytes are destroyed (Lerner, 1971; Copeman, Lewis & Bleehen, 1973). Treatment with either psoralens or steroids appears to promote repigmentation of the skin in a proportion of patients and in the treated areas there is a repopulation of the epidermis with functional melanocytes. These melanocytes seem to spread not only from the pigmented margin of the areas of vitiligo, but from the external hair root sheaths of pigmented hairs in the lesions. Ultrastructural studies on repigmenting vitiliginous skin treated with topical steroids have shown a marked increase in the number of melanocytes that appear to be very active as regards melanogenesis. How these steroids act in promoting the repigmentation of vitiliginous skin is at present unknown. Perhaps in some way they prevent the destruction of melanocytes.

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DISCUSSION

DR S.K.GOOLAMALI (NEWCASTLE): Are you able to predict the response to steroid therapy in patients with vitiligo and is this associated with the presence or absence of antibodies?

DR S.S.BLEEHEN: I think there is a strong probability that young patients with relatively recent onset of vitiligo, in which one can demonstrate some residual melanocytes in their intrafollicular skin, may respond. The antibodies did not help in predicting the outcome.

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The place of intralesional steroid therapy in dermatology

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SUMMARY

Intralesional steroid therapy has a definite, if small, place in dermatology and triamcinolone acetonide is the preparation in most common use. Such therapy may be used, particularly in nail disorders, keloids and hypertrophic scars, cystic acne, and to speed regrowth of hair in localized alopecia areata. Other conditions in which it has a place include cutaneous lupus erythematosus and sarcoidosis. There are some conditions where use is occasionally indicated and yet others where further trial is merited.

Intradermal steroid injection into skin lesions is a commonly practised therapeutic measure. Cortisone acetate and hydrocortisone acetate suspensions were used from the early 1950s prior to the introduction of low solubility preparations of prednisolone, triamcinolone, betamethasone and dexamethasone. The glucocorticoid suspension in most common use at present is triamcinolone acetonide (TA) although triamcinolone hexacetonide (TH) has been introduced more recently. These steroid preparations of low solubility provide depot therapy, being slowly absorbed from the injection site, and not giving rise to systemic effects if used in therapeutic dosage; TH is less soluble than TA.

The total dosage of steroid given at any one attendance depends on the particular disease, the nature of the lesion to be treated and its site, as well as on the age of the patient. The maximum dosage of TA given on any one occasion should not exceed 30 mg and would normally be less. Wilkinson (1972) mentioned that adrenal suppression from intralesional therapy is likely to follow a single injection of more than 75 mg TA. However, it should be noted that Abell & Munro (1973) found the 9 a.m. plasma cortisol in one patient to be 2·7 μg/100 ml the day following 22·5 mg of TA (given for alopecia areata), the pretreatment level being 14·8 μg/100 ml.

The frequency of injections depends on the particular condition to be treated and is often a matter of personal experience. Where improvement in a condition occurs, evidence that this is predominantly a local rather than a systemic effect of the material injected is provided by noting that in the short term the response is only in treated areas.

As with any suspension, it is important that the steroid preparation should be thoroughly shaken before injection so that an accurate concentration is given; if this is not done, blockage of needle, syringe, or jet injector by larger steroid agglomerates may occur.

Before considering some of the diseases that have been treated by intradermal injections, and whether such therapy is worthwhile, it is relevant to describe needle-less injectors briefly. Although a conventional needle and syringe (Bleeker, 1974) or a dental syringe and needle (Wilkinson, 1972) are often adequate, needle-less injectors represent a considerable advance.

The original Porton Jet Injector for giving needle-less injections was described by Moynahan & Bowyer (1965). An improved model called the Port-O-Jet Injector has been available now for some years (Fig. 1) and a small completely hand-operated model (Dermo-Jet or Pan-Jet) is also used. With the Port-O-Jet Injector, accurate volumes of fluid can be injected rapidly (up to thirty injections per min) and with minimal discomfort. Over 500 injections can be given from one loading and no sterilization of the instrument is required between cases. The Injector is spring-loaded and the cocking mechanism is operated via a cable by a foot pedal. The fluid to be injected is suddenly compressed and passes with considerable force through a very fine aperture 1/6000 in (4·23 μm) diameter, the velocity of the fluid jet enabling it to penetrate the skin easily. The nozzle of the instrument is held away from the skin by a guard. The use of a guard prevents spray, positions the nozzle so that the fluid hits the skin at right angles, and prevents injection into deeper tissues. A suspension of TA in normal saline giving a strength 5 mg/ml is used. For sterilization after use, the instrument is cleared of triamcinolone by washing through with sterile water and is then flushed with chlorhexidine 0·5% in 70% industrial methylated spirit. Prior to use it is once again washed through with chlorhexidine in spirit and then should be loaded with this fluid for 10 min to resterilize. Sixty ml glass bottles with rubber and metal seals containing sterile fluid are directly fitted to the gun. The Injector has a calibrated scale and when loaded the volume to be injected (0·05–1·2 ml) is set by rotating a volume selector. The volume of each injection is usually 0·1 ml and approximately thirty 0·1 ml injections will cover an area of 30 cm^2. Injections by injector are given rapidly and cause only slight pain. The use of the high pressure jet injector, rather than a needle, enables easy skin penetration without significant skin damage and the material can be given exactly at the site intended. Most patients are greatly reassured by the fact that there is no needle and children can be treated with this instrument. Prior to injecting an area the skin is cleaned with ether.

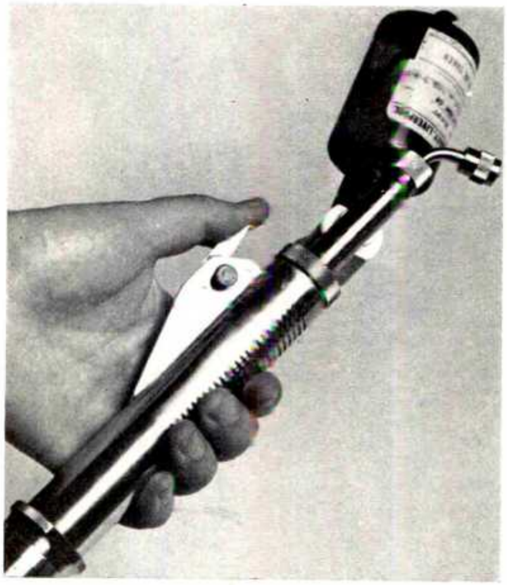

FIGURE 1. Port-O-Jet injector.

Common technical problems with jet injectors include delivery of a lower dose than the calibration indicates and either failure to penetrate the skin or penetration of it too superficially.

Intralesional therapy

There are many skin conditions in which the successful use of intralesional steroids has been reported and a number of these are mentioned in Table 1.

TABLE 1. Conditions in which the successful use of intralesional steroids has been reported

Alopecia areata
Nail disorders
Keloids and hypertrophic scars
Cystic acne vulgaris
Cutaneous lupus erythematosus
Granuloma annulare
Necrobiosis lipoidica
Sarcoidosis
Lichen planus
Lichen simplex
Nodular prurigo
Lichen striatus
Lichen amyloidosus
Pretibial myxoedema
Cutaneous leishmaniasis
Vitiligo
Psoriasis
Herpes zoster
Morphoea
Lichen sclerosus et atrophicus
Pruritus ani
Pyoderma gangrenosum
Aphthous ulcers
Sea-urchin granuloma

Alopecia areata

Successful intralesional steroid therapy using conventional needle and syringe has been described by Orentreich *et al.* (1960), Gombiner & Malkinson (1961) and Porter & Burton (1971). The last showed that both TA and TH produced hair growth in alopecia areata but the hexacetonide seemed to be more effective. Verbov & Abell (1970) assessed seventy-one patients 6 weeks after the first of three consecutive weekly needle-less injections of TA to one or more bald areas. Of these, thirty-eight showed good regrowth of either pigmented or non-pigmented hair and the hair usually remained. In addition, Verbov (1970) compared two groups of patients suffering a first attack of alopecia areata of less than 12 months duration, treated with either conventional therapy or injection therapy. He found that regrowth took longer with conventional therapy and was particularly slow over the beard area. Thus, intralesional steroid therapy is a convenient means of speeding hair regrowth in alopecia areata. Abell & Munro (1973) treated eighty-four patients and found that in localized alopecia a high proportion produced a rapid regrowth of hair but that in established alopecia totalis lasting hair growth was exceptional. It can easily be demonstrated that regrowth is steroid-induced, rather than a placebo effect, by injecting sterile normal saline into an adjacent bald area at the same time as steroid: regrowth over a 6-week period only occurs at the steroid-injected site.

Nail disorders

In the treatment of nail dystrophies, injections into the nail matrix using a needle are too painful to be done without a local anaesthetic (Gerstein, 1962), and a needle-less injector should be used. Abell & Samman (1973a) studied 100 patients with various nail dystrophies including psoriasis (fifty-eight patients), idiopathic onycholysis (fifteen), lichen planus (eleven), and alopecia areata (four). They found that dystrophies involving the nail matrix did well with TA given by injector. Injections were given into the nail matrix through the posterior nail fold. When onycholysis was associated with matrix abnormalities it responded better to treatment than when present as an isolated abnormality. In this series relapse was common but remissions were often long enough to make the treatment worth while. These authors also found injection to be useful in traumatic split nails but in only one of six mucous cysts was there complete recovery. Bleeker (1975) also treated psoriatic nails but after initial improvement found a high relapse rate. Abell & Samman (1973b) reported complete recovery of normal nail growth in five of ten patients with the yellow nail syndrome treated with high-pressure injections of TA into the posterior nail fold.

Keloids and hypertrophic scars

Asboe-Hansen, Brodthagen & Zachariae (1956) and Maguire (1965) treated keloids using needle and syringe with success. Vickers & Stewart (1968) considered intralesional hydrocortisone or triamcinolone to be routine treatment for keloidal scars. Repeated injections over many months, or even years, are often needed for a good response although some lesions, such as post-varicella keloids, may respond rapidly to only one or two injections. If possible, injections should be at intervals of 4–6 weeks but longer intervals may be employed with success. Minkowitz (1967) demonstrated that keloid formation in susceptible patients can be prevented by steroid infiltration of tissues around the surgical scar in the early post-operative period.

Cystic acne

High-pressure jet injection or needle injection of a suspension of hydrocortisone or TA can be very helpful in cystic (abscess) lesions, causing them to subside, albeit sometimes only temporarily. Parish & Witkowski (1967), who treated 234 cysts in twenty-five patients using triamcinolone diacetate or betamethasone acetate-disodium phosphate, obtained better results with a tuberculin syringe and needle than with a Dermo-Jet Injector. These authors suggested that local steroid injections favour the removal of irritating substances causing the acne abscess.

Cutaneous lupus erythematosus

Good results have been reported by Verbov & Abell (1970) and Rowell (1962). Injections should be given every 4–5 weeks but may be more frequent.

Granuloma annulare

Sparrow & Abell (1975) treated forty-five cases of granuloma annulare with either TA or sterile normal saline. Seventy per cent of cases cleared with TA and 44% with saline. The recurrence rate was high but retreatment was usually successful. The authors suggested that treatment with saline should be tried before using triamcinolone.

Necrobiosis lipoidica

Three of five cases that Sparrow & Abell (1975) treated with TA showed gradual resolution. In my experience intralesional TA can abort early lesions but should be avoided in chronic lesions because of the danger of ulceration.

Sarcoidosis

Sullivan *et al.* (1953) used hydrocortisone acetate and cortisone acetate and found that lesions, including papules, responded well to injections but many recurred. Intralesional steroid is useful in the therapy of sarcoid skin lesions but not all types of lesion respond as regularly as some writers have suggested.

Lichen planus

Hypertrophic lesions are often helped by intralesional steroid and nails may be improved (see nail disorders).

Psoriasis

Long-standing resistant localized psoriatic plaques are occasionally treated with intralesional TA. Pelzig & Baer (1960) reported successful treatment in nineteen of twenty patients using needle injections of TA or triamcinolone diacetate subcutaneously, but as superficially as possible, because of the difficulty of intradermal injection into large plaques; only the treated lesions, not others, responded. Posterior nail fold psoriasis is sometimes helped by injections and there is a definite place for intralesional therapy where there is nail involvement (see nail disorders).

Cutaneous leishmaniasis

Intralesional steroid injections are beneficial when used in conjunction with intramuscular antimony in chronic lupoid leishmaniasis.

Pretibial myxoedema

James (1961) reported disappearance of pretibial myxoedema in two patients he treated with intralesional triamcinolone. Lang, Sisson & Lynch (1975) treated nine patients with pretibial myxoedema with intralesional TA (usually 5 mg/ml) giving injections at 3–4 week intervals (a total dose of up to 40 mg was injected at each visit). Complete resolution of myxoedematous plaques was obtained in seven patients and any nodules that recurred responded to further injections.

Lichen sclerosus et atrophicus

Pillsbury & Shelley (1963) recommended intralesional triamcinolone in lichen sclerosus et atrophicus and Wallace (1971) recommended either topical or intralesional steroid, together with local excision or destruction if required, in the conservative treatment of anogenital leukoplakia complicating lichen sclerosus. In the male, intralesional injections may soften sclerotic lesions of balanitis xerotica obliterans and thus reduce phimosis.

Morphoea

Rowell (1972) considered that intralesional triamcinolone (5–10 mg/ml) given at 3–4 week intervals was sometimes justified in localized morphoea but emphasized that in a condition which resolves spontaneously it cannot be justified as a routine measure.

Vitiligo

Kandil (1970) reported repigmentation in vitiligo following intradermal triamcinolone (10 mg/ml). He gave injections at weekly intervals for a maximum of five injections, and treated twenty-six patients with fifty-two patches of vitiligo. Temporary skin atrophy was a problem in his series.

Pyoderma gangrenosum

Moschella (1967) reported healing and loss of pain in pyoderma gangrenosum in a 33-year-old patient with ulcerative colitis treated with intralesional triamcinolone diacetate. The leg ulcer received 48 mg triamcinolone over a 2-week period; the patient's bowel symptoms did not improve in a 3-week period from the start of intralesional steroid but the ulcer had completely healed in this time. Gardner & Acker (1972) in their 71-year-old rheumatoid arthritic patient with many pyoderma gangrenosum lesions used large doses of TA (40 mg/ml) and injected around the perimeter of lesions. Healing occurred rapidly, each individual lesion only receiving one injection initially, although some required a further injection in the following months. The patient received a total of 600 mg TA over a $5\frac{1}{2}$-month period, equivalent to less than 4 mg prednisone daily.

Herpes zoster

Fifteen patients (thirteen were over the age of 54 years) with herpes zoster (eight patients) or post-zoster neuralgia (seven patients) were treated by Epstein (1970) with a triamcinolone–procaine solution injected subcutaneously (to prevent skin atrophy) into the areas of maximum discomfort; injections were often given daily. The results were reported as excellent in controlling the pain and in eradicating the cutaneous manifestations. The author emphasized the necessity of using the combination of drugs rather than either alone. In one patient triamcinolone and procaine were used separately with no benefit. It would seem worth while repeating this study using TA alone given by needle-less injector to judge whether it has any long-term beneficial effect.

Complications of intralesional therapy

Bleeding may occur but oozing is easily controlled. Sepsis is uncommon. Temporary skin atrophy is not uncommon, tends to be proportional to the concentration of drug used, and related to the site, depth, and the number of injections given at a particular site. Tanenbaum & Becker (1964) reported a case of permanent atrophic scarring occurring in two psoriatic plaques after intralesional triamcinolone diacetate (25 mg/ml). Patients with lupus erythematosus profundus treated with intralesional triamcinolone who developed superficial ulceration (Verbov & Abell, 1970) and abscess formation (Rowell, 1962) have been reported. Special care is advised in the use of needle-less jet injectors when injecting atrophic skin lesions over cheeks and lips because perforation of the underlying mucosa is a possible hazard (Cohen & Lerner, 1972); using a needle and syringe here may allow the depth of penetration to be more easily controlled.

DISCUSSION

Intralesional steroid therapy is a safe and straightforward form of therapy if used with care. I believe that it does have a place in dermatology but only in a few conditions and then only in selected cases. Injection has become easier and more accurate with the appearance of high-pressure jet injectors. Although conventional needle and syringe are adequate, needle-less injectors show a particular advantage over them as an efficient means of delivering steroid to nail matrix and to scar tissue.

In some conditions, such as localized alopecia areata, granuloma annulare and cystic acne, intralesional steroid can hasten resolution that would in most cases occur naturally. However, such treatment can often be of real benefit to the patient. In addition, it is probable that steroid injections may in some cases actually stimulate natural resolution to take place.

Many keloids and hypertrophic scars do not resolve spontaneously and intralesional steroid has an important indication here.

Recurrence of an apparently resolved lesion is often a problem but reinjection sometimes results in a permanent cure. Intralesional therapy has a place in the treatment of some nail disorders where some form of effective treatment is urgently required and no other suitable alternative at present exists.

Plaques of discoid lupus erythematosus can be effectively treated with intralesional therapy.

Extensive alopecia areata, including totalis and universalis, is a contraindication in my opinion; if any regrowth does occur it is usually poor and only temporary. Time and time again in the past I have noticed patients with extensive alopecia who have come to rely on intralesional therapy, convinced that it is regrowing their hair and becoming addicted to it; one must not be persuaded to use intralesional steroid in such patients.

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DISCUSSION

DR C.DUPONT (DUBLIN): How do you think that, from the point of view of efficiency, intralesional triamcinolone injections compare with the long-term use of clobetasol propionate with polythene occlusion?

DR J.VERBOV: I have never used clobetasol propionate with polythene occlusion.

DR H.BAKER (LONDON): Blindness apparently induced by needle injection of steroid in alopecia areata has been referred to by Orentreich of New York. He made what could be the very important point: that this seems to have happened in patients where the needle has been inserted into the skin, withdrawn and pushed in another direction to spare the patient the discomfort of multiple punctures, and that possibly the needle has gone into a small artery, is withdrawn, the artery being pulled along and the needle staying in the vessel; each injection is given into the same vessel and this is responsible for the embolus. I think two or three more cases of this devastating complication have now been reported.

DR J.VERBOV: This is a very important point. Orentreich was one of the pioneers of intralesional therapy, and this rare complication has been reported (Baban, 1964).

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Steroids and oral disease

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SUMMARY

Small doses of topical corticosteroids have been widely used in severe varieties of recurrent aphthous ulcers (RAU), erosive lichen planus, benign mucous membrane pemphigoid and erythema multiforme. The rationale for treatment of RAU with steroids is that the early histological changes are those of delayed hypersensitivity, and that a significant increase in lymphocytes sensitized to oral mucosa occurs during the prodromal phase, reaching a maximum 2–3 days after the ulcers have appeared (Lehner, 1967, 1969a). The hypothesis that RAU is an auto-immune condition has been substantiated by the cytotoxicity of lymphocytes from patients with RAU to epithelial cells (Dolby, 1969; Rogers, Mitchell Sams & Shorter, 1974).

Four topical steroids have been used in oral diseases, and except for the double-blind trial of hydrocortisone sodium succinate (Cooke & Armitage, 1960), much of the evidence for the efficacy of the other steroids is empirical or from uncontrolled studies; this includes triamcinolone in orabase (0·1%) and betamethasone valerate (0·1 mg tablet). The efficacy of topical steroids can be greatly increased if they are administered during the prodromal phase of ulceration (Lehner, 1969b), when lymphocyte activity is at its maximum.

Adreno-corticosteroid suppression can result from some topical steroids; 0·4 mg of betamethasone disodium-phosphate (no longer available) induces adrenal suppression in practically all patients but the same dose of betamethasone valerate and therapeutic doses of triamcinolone or hydrocortisone do not affect the plasma cortisol level (Lehner & Lyne, 1969, 1970). The therapeutic doses of betamethasone valerate, triamcinolone or hydrocortisone can be exceeded three times without impairing adrenal function. However, some of these steroids can induce acute pseudo-membranous candidiasis without any changes in the plasma cortisol level (Lehner & Ward, 1970). This was found in five of twenty-two (23%) patients using topical betamethasone valerate, and their IgG and IgA fluorescent antibody titres were significantly increased before infection, suggesting previous candidiasis and therefore a predisposition to this infection. An increased prevalence of oral candidiasis (45–75%) and precipitating antibodies (IgG) have been recorded recently with beclomethasone dipropionate (Turner-Warwick, Clark & Fox, 1974).

Topical corticosteroids play an important part in the management of oral ulcerative lesions, provided adequate attention has been paid to the differential diagnosis, correct timing and application of the drugs. Most of the ulcers can be controlled, though not cured by topical steroids, but very occasionally systemic steroids have to be used.

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DISCUSSION

DR R.C.D.STAUGHTON (LONDON): Is there any evidence to suggest that prophylaxis with antifungal treatment before the initiation of treatment of painful local oral conditions with steroid can prevent the emergence of the secondary problem of oral candidiasis?

PROF. T.LEHNER: I think there is clinical evidence. Any of our patients, especially those with lichen planus, who are liable to develop candidiasis on topical steroids are usually given topical nystatin or amphotericin B. We find amphotericin B, 100 mg twice daily more suitable.

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The use and interpretation of tests for steroid impaired hypothalamic–pituitary–adrenocortical function

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INTRODUCTION

The problem of investigating and treating disorders of the hypothalamic–pituitary–adrenal (HPA) axis is one which is becoming increasingly familiar to physicians in all specialities—partly because of the widespread use of corticosteroid preparations which interfere with the axis. Diagnostic and investigational techniques are better developed than ever before and there is now a wide range of dynamic test procedures to assess the various components of the axis and of the physiological mechanisms which control it. Furthermore, the increasing availability of radio-immunoassays for adrenocorticotrophin (ACTH) and of binding and other assays for cortisol, applicable to both plasma and urine, has ensured that such tests can be performed in most large hospitals.

Physiological considerations

It is important to remember that the adrenal glands function as part of a complex which also involves the brain, including the hypothalamus, and the anterior pituitary. The anterior pituitary hormone, ACTH, is the only physiological substance which will alter the rate of biosynthesis and secretion of cortisol. In its absence marked diminution of cortisol secretion occurs and atrophy ensues. The secretion of aldosterone by the adrenal cortex is not, however, markedly impaired since this is controlled primarily by the renin-angiotensin mechanism. The pituitary which lies within the sella turcica is separated from the brain by the fibrous diaphragma sellae, through which pass neural connections to the posterior pituitary and vascular connections from the hypothalamus to the entire gland. The hypothalamus has an important endocrine role in controlling the anterior pituitary. Thus, nuclei within the hypothalamus secrete hormones which reach the anterior pituitary via a hypothalamic–hypophyseal portal system. One of these, corticotrophin releasing factor (CRF), plays a dominant role in controlling the rate of ACTH secretion and in its absence, due either to hypothalamic dysfunction or to interruption of the vascular pathways, ACTH, and therefore cortisol secretion is markedly impaired. The secretion of CRF is, in turn, influenced by neural impulses from the brain and a number of stimuli such as trauma, noise and fever stimulate the HPA axis by influencing the release of CRF.

Clinical test procedures are based on an understanding of the mechanisms which control the activity of this system, and a brief description of these mechanisms is a necessary prelude to any consideration of the available dynamic tests.

Three major mechanisms operate, namely a negative feedback mechanism, a mechanism responsible for the circadian rhythm of the axis, and a stress-control mechanism. At any particular time, homeostasis is maintained by a negative feedback mechanism whereby an increase in circulating cortisol inhibits ACTH secretion, probably via an effect on the rate of CRF secretion by the hypothalamus. Conversely, a fall in circulating cortisol levels stimulates CRF release and, thereby, ACTH and cortisol secretion. This is often referred to as the 'long feedback loop' and there is evidence which indicates the presence of a 'short feedback loop' whereby ACTH affects CRF secretion, also in a reciprocal manner. An additional homeostatic control mechanism explains the well-marked circadian variation in cortisol secretion. This is a function of the subject's sleep–wake cycle and normally plasma cortisol levels are at a minimum at, or soon after, midnight. Thereafter circulating cortisol levels gradually rise to reach a maximum between about 6 and 8 a.m. and then fall again slowly. A third independent mechanism operates in response to a variety of stressful situations and results in a temporary elevation of circulating cortisol levels. There is considerable clinical evidence emphasizing the physiological importance of this 'stress' mechanism. Thus some patients with adrenocortical insufficiency remain well until they encounter a situation, such as surgery or infection, which may precipitate an acute adrenal crisis. Similarly it is recognized that patients with adrenal insufficiency require increased steroid replacement therapy if subjected to stress.

Procedures used in the investigation of HPA function

The various diagnostic procedures which are employed to investigate the functional integrity of the HPA axis are summarized in Table 1. Some, such as the determination of basal plasma and urinary corticosteroid levels, provide information about basal activity but give no indication of the functional reserve of the system. In addition to maintaining an adequate rate of cortisol secretion at rest, it is essential that the axis operates as an integrated whole, with sufficient reserve capacity to enable an adequate increase in circulating cortisol levels during periods of stress. Tests based on the plasma or urinary corticosteroid response to exogenous ACTH or its synthetic analogues (such as Synacthen,

TABLE 1. Diagnostic procedures employed in the assessment of the hypothalamic–pituitary–adrenocortical system*

Determination or procedure	Control mechanism and/or component assessed
Basal plasma corticosteroid levels Basal plasma ACTH levels Basal urinary corticosteroid excretion Cortisol secretion rate Urinary free cortisol	Basal homeostasis
Plasma corticosteroid levels throughout 24 h	Circadian control mechanism
Response to dexamethasone Response to Metapirone	Functional integrity of negative feedback control
Response to pyrogen† Response to insulin-induced hypoglycaemia Response to vasopressin†	Functional integrity of components involved in stress control mechanism
Adrenocortical response to exogenous corticotrophin, Synacthen depot (or ACTH)	Functional integrity of adrenal cortex
Half-life of cortisol	Ability of liver to clear cortisol

* It must be emphasized that this is a simplified scheme to indicate broad principles.
† Tests now seldom employed.

which comprises the N-terminal 24 amino acids of the parent molecule) assess the reserve capacity of the adrenal cortex and supply quantitative data concerning the presence and degree of adrenal atrophy.

Another test of particular value in the investigation of the corticosteroid-treated patient is to determine the plasma cortisol response to insulin-induced hypoglycaemia. This assesses all the components of the HPA axis and, most important from a clinical standpoint, the integrity of the stress-control mechanism. The test is safe provided it is not performed on patients with a history of epilepsy or myocardial insufficiency; the dose of insulin is based on body weight (usually 0·15 units/kg) and is given intravenously; a doctor is present throughout; glucose is available on the rare occasions when side-effects are severe and the patient eats a carbohydrate-containing meal at the conclusion of the test. In the few patients in whom insulin is contra-indicated, a subcutaneous or intravenous injection of 1 mg of glucagon can be given, blood samples for cortisol assay being taken at half-hourly intervals for 4 h.

None of the remaining procedures has any major role in the investigation of the corticosteroid-treated subject. Thus the assay of urinary oxo- and oxogenic steroids (the latter representing the main metabolites of cortisol) is complex and time-consuming and the accurate collection of urine over a 24-h period is notoriously difficult. Tests based on the urinary oxogenic response to the 11 β-hydroxylase inhibitor, Metapirone, assess only the negative feedback mechanism and there is no place for the dexamethasone suppression test in this group. Finally the unpleasant side-effects associated with tests based on the administration of pyrogen and vasopressin are such that their use is not justified.

The effects of corticosteroid administration on the HPA axis

The administration of corticosteroids for therapeutic purposes is by far the most common cause of secondary adrenocortical insufficiency. Thus the administration of natural or synthetic corticosteroids (such as cortisol, cortisone, prednisolone, prednisone, triamcinolone, dexamethasone, and betamethasone) will, almost invariably, cause some degree of adrenal atrophy. In general, the degree of suppression of the HPA axis is related both to the daily dose of steroid given and to the duration of its administration. Although some patients are more susceptible than others the administration of 2·5 mg of prednisolone daily causes little, if any, suppression while, at the other end of the scale, patients receiving 20 mg or more daily rapidly develop a considerable degree of adrenal atrophy.

There is also evidence that the time at which corticosteroid therapy is given influences markedly the degree of HPA suppression. Thus the adrenal atrophy caused by prednisolone and other corticosteroids is least when the main dose is given on rising and most marked when the drug is taken last thing at night. This would be expected on the basis of the circadian rhythm since a late evening dose suppresses the normal early morning activation of the axis. There is also evidence that giving large doses of corticosteroids separated by intervals of 2 or 3 days causes less suppression than the administration of an equivalent amount given once or more daily.

Exogenous corticosteroids exert their effect on the HPA axis by suppressing CRF and thereby ACTH secretion via the negative feedback control mechanism. Since ACTH, in addition to stimulating an adequate rate of cortisol secretion, is also necessary to maintain the size of the adrenal, its absence results in adrenocortical atrophy. It should be noted that biochemical (namely an inability of the adrenals to increase their rate of cortisol synthesis) precedes the presence of anatomical atrophy. After stopping therapy it may take several weeks for the HPA axis to recover fully. Because circulating ACTH levels are elevated during the latter part of this recovery period it would seem probable that the adrenal atrophy takes longer to recover than does any associated defect at the pituitary or hypothalamic level.

The use of dynamic test procedures in the steroid-treated patient

Using the various tests now available, it is possible to examine the functional state of the HPA axis in the corticosteroid-treated patient and to form an accurate opinion of the degree of suppression which has occurred. Before doing so, however, it is important to be clear why such an investigation is to be carried out, since, if it is the intention to continue steroid therapy, nothing of practical value with regard to treatment is achieved by such studies.

In clinical practice the most common situations in which HPA assessment is required are in patients who are requiring surgery, in patients whose steroid therapy has been terminated if the physician wishes to ensure that HPA function has been restored and in the patient who is receiving or has received corticosteroids and who presents with signs and symptoms suggestive of an acute adrenal crisis. In our opinion, as stated earlier, the most valuable procedures for these purposes are the determination of basal circulating cortisol levels and their response to Synacthen stimulation and to insulin-induced hypoglycaemia. A normal response to these two tests indicates that HPA function is intact.

HPA function during and following steroid withdrawal. The clinician need not anticipate problems on discontinuing corticosteroid therapy and tests of HPA function are very seldom necessary. Thus the occurrence of severe signs and symptoms following steroid withdrawal is extremely uncommon and most cases reported in the literature are insufficiently documented to discount other disorders which produce a similar clinical picture. None the less, steroids should be withdrawn slowly both to avoid an acute exacerbation of the disorder for which they were given and to enable sufficient time for the recovery of the HPA axis. One successful schedule is to convert part of the dose to the 1 mg prednisolone tablets employed in paediatric practice (since even half an adult tablet is 2·5 mg) and limit the reduction to 1 mg every 3rd day with, as explained earlier, most of the dose being taken on rising.

Although ACTH therapy causes less HPA suppression than does corticosteroid administration, under no circumstances should it be given during or at the end of a course of corticosteroids. Thus while the exogenous ACTH will rapidly reverse the adrenal atrophy, its administration further impairs hypothalamic and/or pituitary function.

The steroid-treated patient requiring surgery. A second problem is the steroid-treated subject who requires surgery or who is subjected to other stressful situations such as infection or a fracture. There is now adequate information available to set out certain guidelines. Within 3 months of stopping corticosteroids, HPA function is fully recovered and there is no need to perform any investigations. In patients receiving corticosteroids, or in those who are within 3 months of their discontinuation, it is wise to determine the plasma cortisol response to insulin-induced hypoglycaemia. If normal, surgery can be undertaken without steroid cover. If impaired, a satisfactory regime is to give 100 mg of cortisol by intramuscular injection at the same time as the premedication. This is the only cover required for simple procedures such as endoscopy, while further 100 mg injections 6-hourly for 24 h are sufficient for minor surgery and their continuation for 72 h will cover major operations such as gastrectomy or hip replacement. The normal dose can then be given by mouth (or by injection if oral administration is not possible) and only if the patient is severely stressed is it necessary to give larger than normal doses.

The patient presenting with suspected acute adrenal crisis. Another situation which merits comment is that of the corticosteroid-treated patient who presents with signs and symptoms suggesting an acute adrenal crisis. This is a life-threatening situation and treatment must not, therefore, be delayed. Blood should be taken immediately, for subsequent plasma corticosteroid assay, and vigorous therapy instituted with intravenous steroids and, if necessary, fluid replacement, when waiting for the result. Tests of adrenal function are just as easily made after this treatment has been started provided prednisolone is employed for replacement purposes and not cortisol (since prednisolone does not interfere with most of the available assays). An ACTH-stimulation test should be carried out as soon as possible, bearing in mind that steroid replacement therapy will produce some depression of adrenal function. Confirmation or rejection of the diagnosis of an acute adrenal crisis can readily be obtained from knowledge of the initial plasma corticosteroid level and the subsequent ACTH test.

It must be remembered that improvement in the patient's condition following intravenous steroid administration is not evidence that the collapse was due to an acute adrenal crisis, since improvement can also occur with collapse due to such causes as *E. coli* septicaemia or acute blood loss. Indeed these latter two conditions are much more common and can give a clinical picture identical with acute adrenal insufficiency.

CONCLUSION

The management of the steroid-treated patient is relatively straightforward and dynamic tests of HPA function are seldom required. Resort to a wide range of such tests is unpleasant for the patient and imposes an unnecessary analytical load on the laboratory.

The procedures of most value are to determine the basal circulating cortisol level and its response to exogenous ACTH (to assess adrenocortical reserve) and to insulin-induced hypoglycaemia (to assess the integrity of the entire axis and the stress response mechanism). The most common instances in which these procedures are required are when the physician wishes to assess HPA function in patients following steroid withdrawal or in those still receiving steroids who require surgery or in such patients who present with signs and symptoms suggesting an acute adrenal crisis.

DISCUSSION

DR A.CARRUTHERS (LONDON): It has been a clinical impression that some of the patients we see treated with a systemic corticosteroid respond differently from others.

PROF. J.LANDON: People vary considerably in their rate of absorption. Some absorb less than others, and it is not surprising that some people have more efficient catabolic systems than others. The half-life of cortisol varies from 70 min in some people to 110 min. If you do something which changes the half-life, you must change your steroid dose. Remarkably little has been done on the metabolism of steroids in different situations compared with work on their secretion.

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The effect of percutaneously absorbed steroids on hypothalamic–pituitary–adrenal function after intensive use in in-patients*

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SUMMARY

Hypothalamic–pituitary–adrenal (HPA) axis function has been monitored in adults and children who required intensive treatment of their skin disease with topical corticosteroid preparations while in hospital. Evidence of mild suppression of the HPA axis was seen in adults when the more potent topical steroids were used, but recovery of function was rapid when the intensive treatment ceased. In children suppression was still present in twelve of sixteen cases on the 2nd day after treatment with 0·1% betamethasone 17-valerate ointment had stopped, yet in nine cases treated in a comparable manner with 1% hydrocortisone acetate ointment, there was no evidence of impaired HPA axis function.

Topical corticosteroids are the most widely prescribed medications for dermatological disease because they are so efficient in treating inflammatory and epidermal proliferative diseases. In their usefulness lies one of their dangers, namely, that when great quantities of extremely potent drugs are being prescribed by physicians, versed and non-versed in their side-effects, and when they are being used by individuals for whom they have not been prescribed but have become available in their homes as the result of other people's prescriptions, the potential for misuse of the preparations is high.

It reflects credit on the many potent drugs being used by the medical profession today, that the side-effects of gross overdosage or misuse may commonly have such little dangerous effect on our patients. Topical corticosteroids are no exception to this principle and reports which appear in the medical press of gross systemic or local changes in patients being treated with these useful medications, commonly fail to emphasize that it is not the correct use of the drug which has caused the problem, but gross misuse, reflecting discredit on the physicians who prescribed the preparation.

There is a school of opinion in the United States of America currently lobbying for the 'over the counter' sale of 0·5% hydrocortisone ointment without prescription, and although at first sight this may be viewed with concern, on deliberation one has to accept that with a little advice printed on the tube such as 'Consult your dermatologist if the rash does not respond in 1 month' or 'Avoid the use of more than 30 g of ointment per week', the real risk of serious health hazard must be minimal, and certainly less than with such preparations as opiate-containing cough medicines or aspirin, phenacetin or amidopyrin analgesics which have been freely available for years.

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* Some of the work reported formed part of an M.D. Thesis, University of London 1975.

Investigations have been undertaken in both adults and children to determine the effect of very intensive high-dose topical corticosteroid therapy on hospital in-patients in order to quantify the degree to which absorption may cause suppression of the hypothalamic–pituitary–adrenal (HPA) axis.

Methods and investigation details in adult cases

In order to demonstrate suppression of the HPA axis if the possibility of this was likely in ordinary clinical use, the individuals chosen for study were suffering from widespread skin disease, usually involving 60% or more of the body surface. The patients were treated intensively in hospital with 30 g of topical corticosteroid ointment applied daily, and the skin was occluded with a polyethylene 'polythene' suit for 20 out of 24 h each day. Schlagel & Sanborn (1964) had demonstrated that 30 g of an emollient allowed a sparing application all over the body, of an average-sized adult, and this was confirmed in the present study.

Patients collected their urine over 24-h periods throughout admission, and samples of blood for cortisol estimation were withdrawn at 9.00 a.m. each day. The urine was analysed for 17-hydroxycorticosteroid (17-OHCS) levels (Norymberski and colleagues method of the M.R.C. Committee on Clinical Endocrinology, 1963) and the blood cortisol estimations were performed by the Mattingly (1962) method.

A pretreatment period usually lasting 3 days enabled baseline readings to be obtained, and during this period, and again during the post-treatment period, insulin stress tests (Greenwood, Landon & Stamp, 1966) were performed. The corticosteroid ointment treatment period lasted 14 days, and the integrity of HPA axis function was tested on the 2nd post-treatment day by an insulin-stress test (IST) and on the 3rd post-treatment day by a metyrapone test (Liddle *et al.*, 1959).

In certain individuals additional tests were performed in order to document changes in the HPA axis function more efficiently over the period of the investigation.

The normal plasma cortisol range for the resting subject at 9.00 a.m. is 7–25 µg/100 ml with a mean level about 8 µg/100 ml. A normal response to the insulin-stress test is an incremental rise, from the resting level of plasma cortisol, of at least 7·5 µg/100 ml to 20 µg/100 ml when the blood sugar has dropped to 40 mg/100 ml or below. The normal value of urinary 17-OHCS is 4–20 mg/24 h, the normal response to a metyrapone test being a rise of 10 mg/24 h above the level for the control days.

Results in adult cases

The result of an individual case treated with Betnovate ointment (0·1% betamethasone 17-valerate) is shown in Fig. 1. The high plasma-cortisol levels soon after admission reflect the stressful state of this ill patient. The plasma-cortisol readings fall rapidly once the steroid applications start, yet when they cease, recovery of cortisol level was seen by the 3rd day. The high urinary 17-OHCS pre- and post-treatment reflect the normal response to metyrapone before treatment and on the 3rd post-treatment day. The insulin-stress test on the 2nd post-treatment day, however, reflects an abnormal response even though HPA axis function is recovering rapidly.

Another interesting example is shown in Fig. 2, where the patient had clearly been using potent topical preparations prior to admission although she had denied this. The minimal response to insulin stress on the 1st day of the study and the rapid rise in blood cortisol readings in the pretreatment period obviously represent recovery from the HPA axis suppression which had occurred prior to admission. The treatment with Locorten ointment (flumethasone pivalate 0·02%) gave no fall in plasma cortisol levels though the urinary 17-OHCS dropped to 4·3 mg/24 h on day 9. The IST on

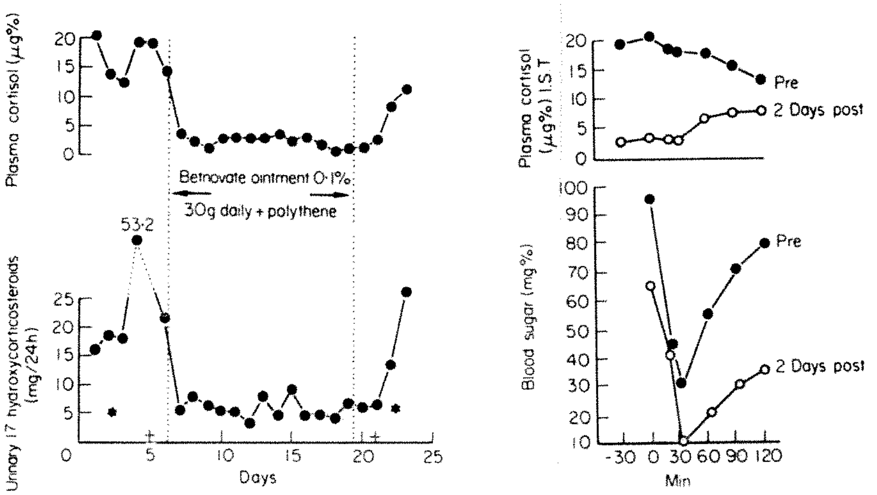

FIGURE 1. Mr A.H., aged 42 years, atopic eczema—90% surface, treated with Betnovate ointment. * Metyrapone; + IST.

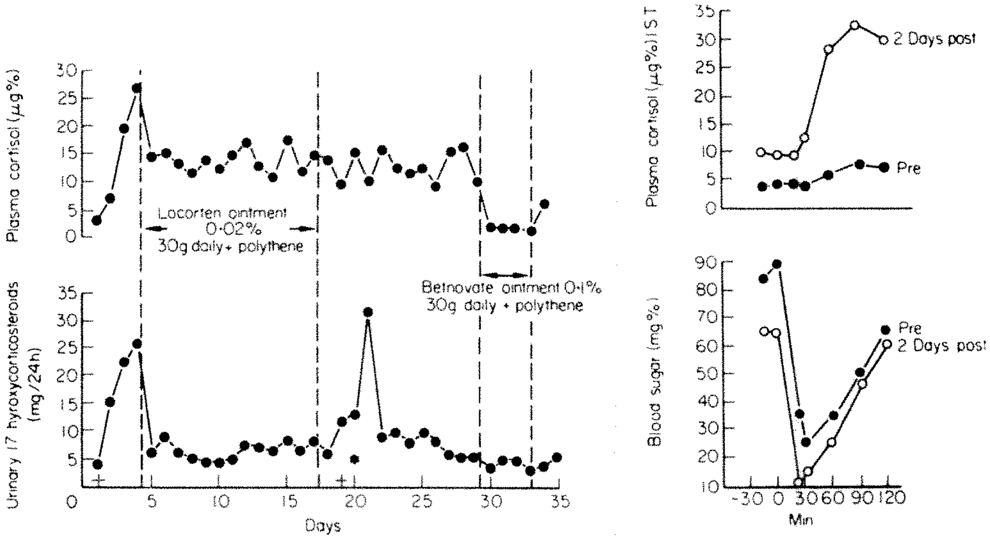

FIGURE 2. Mrs L.N., aged 67 years, constitutional eczema—70% surface, treated with Locorten ointment and later with Betnovate ointment. * Metyrapone; + IST.

the 2nd post-treatment day gave a brisk and normal response and similarly the metyrapone test the following day gave evidence of normal HPA axis function. However, the patient's eczema relapsed and treatment with Betnovate ointment was started on day 29, with immediate evidence of HPA axis suppression and incidentally, rapid clinical control of her disease.

Studies of HPA axis function associated with topical corticosteroid or systemic steroid therapy indicate clearly that there is a marked individual variation between patients in their ability to develop and recover from HPA axis suppression. For this reason it is helpful to study the results of patients in groups which may gave a more accurate assessment of the problem.

Fig. 3 shows the result of six patients treated with Betnovate ointment. The mean values ± s.e. are

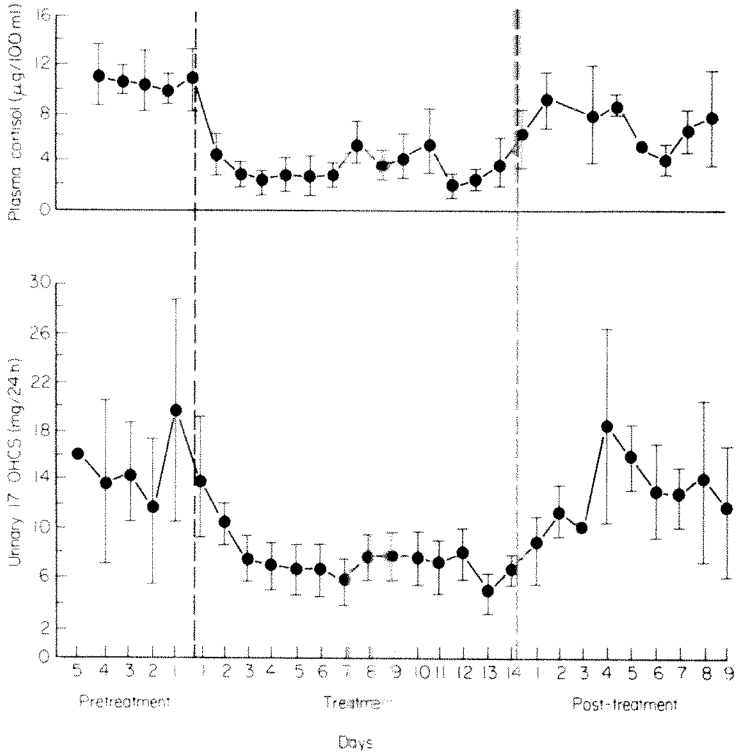

FIGURE 3. Betnovate ointment group, six cases, 30 g daily + polythene, mean ± s.e.

plotted. The pattern of fall in plasma cortisol and urinary 17-OHCS during the treatment period is clearly seen with rapid recovery within a few days of stopping treatment.

Of the six patients in this group the insulin stress tests on the 2nd post-treatment day were normal in five of the six, this later became normal by the 8th day. The metyrapone test was normal in all on the 3rd post-treatment day.

This result compares with Fig. 4 in which the values for four cases treated with Locoid cream (hydrocortisone butyrate 0·1%) are shown. It is important to emphasize that in this group the cream preparation was used (it is probably absorbed less well than ointments), and that 30 g were applied twice daily instead of the usual 30 g once daily. The pattern of reduction in plasma cortisol levels is similar in the Betnovate ointment and Locoid cream groups, the comparison of pretreatment with treatment levels in both being significantly reduced ($P<0{\cdot}001$) though the comparable urinary 17-OHCS showed significant reduction in the Betnovate ointment group ($P<0{\cdot}02$) but not in the Locoid cream group ($P>0{\cdot}50$). The insulin-stress tests on the 2nd post-treatment day were abnormal in three of the four Locoid cream cases, and the metyrapone test was also abnormal in two of the four.

Locorten ointment (flumethasone pivalate 0·02%) was applied to the cases shown in Fig. 5. The differences between pretreatment and treatment levels were not significantly different for either plasma cortisol ($P>0{\cdot}10$) or urinary 17-OHCS ($P>0{\cdot}10$) mean values. The post-treatment insulin-stress tests were normal in all the cases. The metyrapone test was abnormal in one case, which probably reflected an incomplete 24 h collection of urine, but was normal in the other two.

This compares impressively with the cases studied in Fig. 6 who were treated with Locasalen ointment. This preparation is identical to Locorten ointment except that it contains 2% salicylic acid

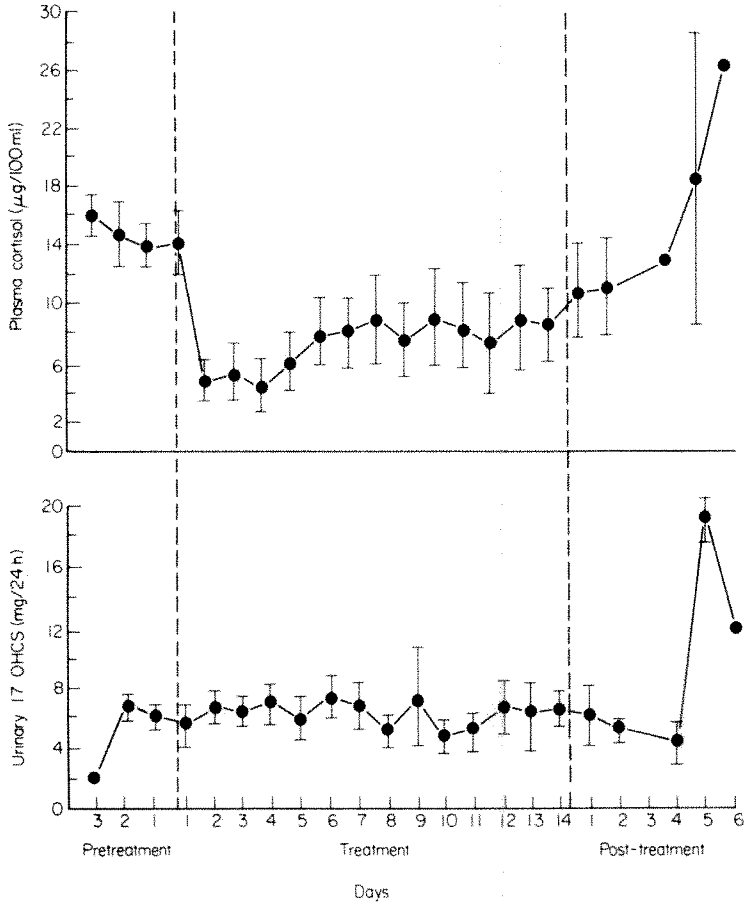

FIGURE 4. Hydrocortisone butyrate cream (Locoid) group, four cases, mean ± s.e.

which greatly increases its penetration and clinical potency. This difference is reflected in the results of five cases where the levels of plasma cortisol ($P < 0{\cdot}001$) and of urinary 17-OHCS ($P < 0{\cdot}001$) clearly fall to significant levels during treatment compared with pre-treatment. The post-treatment insulin-stress tests were abnormal in three, and the metyrapone test abnormal in two cases.

The influence of polyethylene occlusion of the skin during treatment with potent corticosteroids is shown in Fig. 7 where Metosyn cream (fluocinonide 0·05%) was used in three patients. The polyethylene suit resulted in absorption such that the plasma cortisol levels fell significantly during treatment ($P < 0{\cdot}001$) and similarly, the urinary 17-OHCS levels fell ($P < 0{\cdot}02$). Either the post-treatment insulin-stress test or metyrapone test was abnormal in each case.

This contrasts with a comparable group using Metosyn cream without polyethylene suits, the skin being covered only with stockinette to avoid the cream being rubbed off. These cases, Fig. 8, showed a reduction in the plasma cortisol levels during treatment as part of the pattern seen when patients relax in hospital, but the mean value was 16·0 μg/100 ml (± s.e. 0·74) and clearly remained within the physiological range as did the urinary 17-OHCS (mean 5·8 ± s.e. 0·42). The post-treatment insulin-stress tests were normal in all the cases.

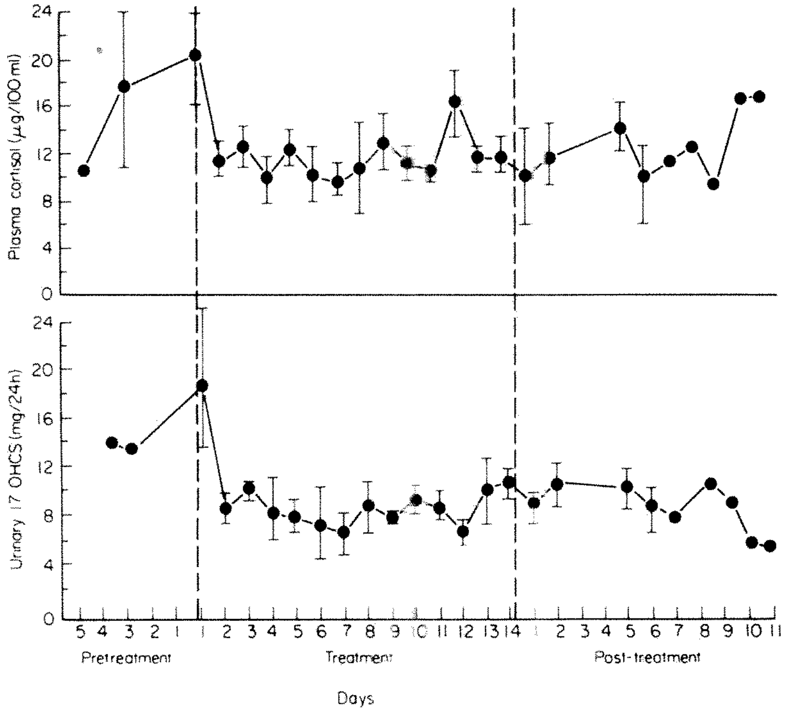

FIGURE 5. Locorten ointment group, three cases, 30 g daily + polythene, mean ± s.e.

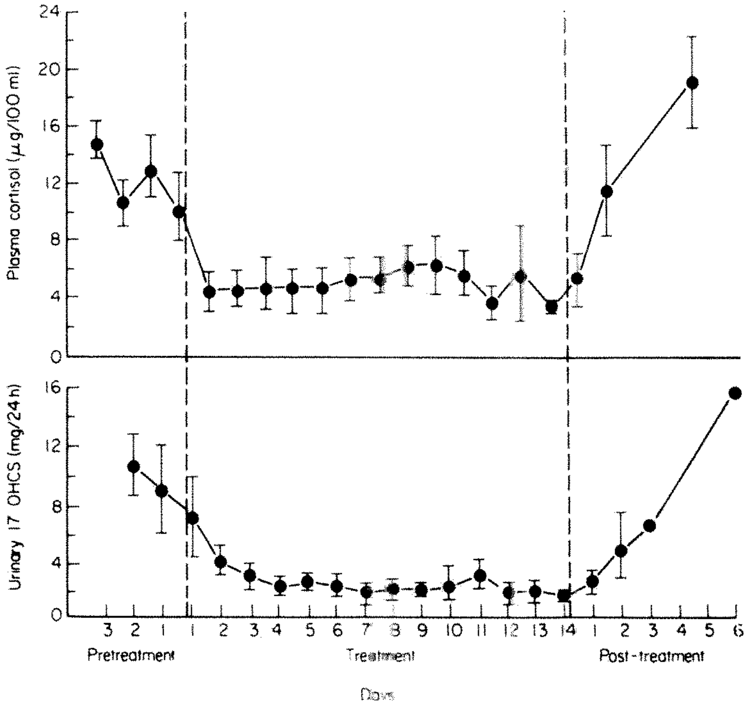

FIGURE 6. Locasalen ointment group, five cases, 30 g daily + polythene, mean ± s.e.

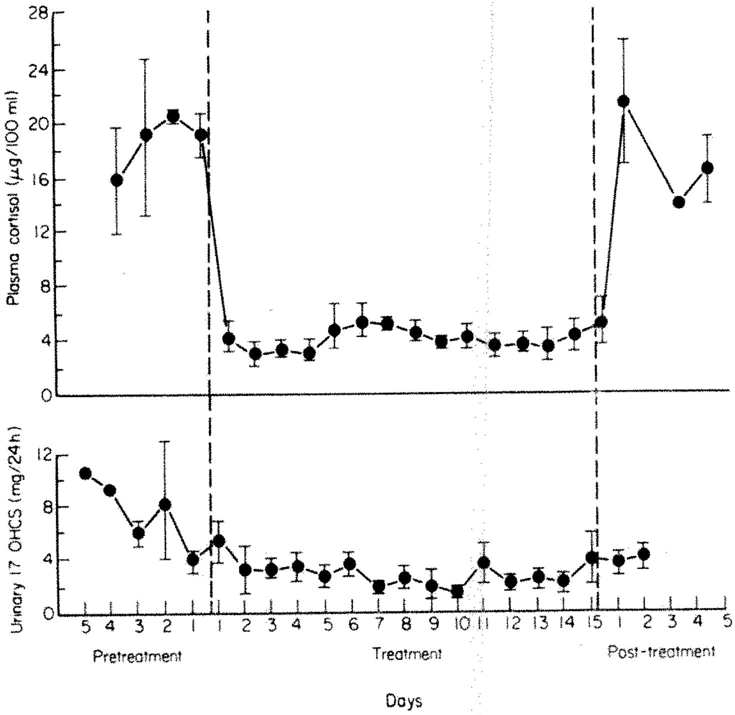

FIGURE 7. Metosyn cream group, three cases with 'polythene', 30 g daily, mean ± s.e.

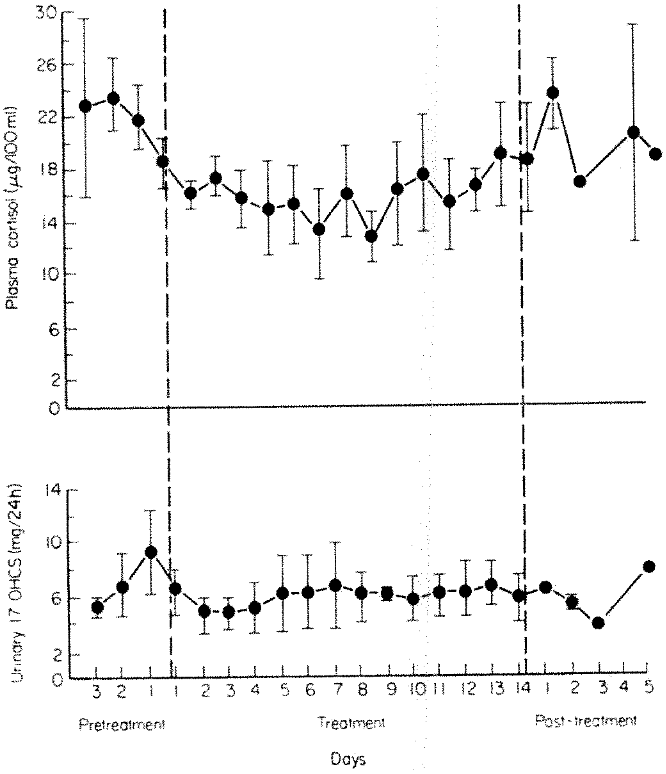

FIGURE 8. Metosyn cream group, three cases with stockinette, 30 g daily, mean ± s.e.

Methods and investigation details in children

Two groups of children were studied, the first was composed of eighteen individuals with a mean age of 3·9 years (±s.d. 2·6). The children suffered from eczema (except one boy who had psoriasis) and the mean surface area involved was 50·0% (±s.d. 16·5). The skin was treated twice daily with Betnovate ointment and then covered with stockinette. The quantity used each day was varied according to the size of the child and the surface area covered by eruption. The majority of cases received 15 g/day divided into two applications, the mean quantity being 12·9 g (±s.d. 3·5).

For ethical reasons it was clearly not possible to perform the many complicated tests of HPA axis function which had been used in the adults, and the investigations were confined to plasma cortisol samples every 3rd or 4th day, and at the end of treatment a tetracosactrin test (short Synacthen test) (Wood *et al.*, 1965) was performed on the 2nd post-treatment day. In this test a blood sample is taken for resting plasma cortisol, the tetracosactrin is injected intramuscularly in a dose of 0·125 mg and after 30 min a further sample for plasma cortisol is collected. In a normal adult the rise in cortisol level should be at least 7·5 μg/100 ml and reach a level of 20 μg/100 ml or more.

In the second group of infants a comparable study was performed but 1% hydrocortisone acetate ointment was used instead of Betnovate ointment. Because endogenous and exogenous hydrocortisone cannot be distinguished by the Mattingly (1962) method for the estimation of plasma-cortisol (hydrocortisone), tetracosactrin tests were performed prior to starting treatment and on the 2nd post-treatment day. The ages and severity of the eczema in this group of children were similar to the Betnovate group; the mean daily quantity of ointment used was 18·7 g (±s.d. 6·5).

Results in children

Prior to treatment the resting plasma mean cortisol value was 16·0 μg/100 ml in the Betnovate treated group and rapidly fell during treatment to 1·8 μg/100 ml on the 6th day; thereafter it gradually returned to a more normal level of 7·8 μg/100 ml on day 15.

On the 2nd post-treatment day the mean plasma cortisol resting value was 8·0 μg/100 ml (±s.d. 4·7) and the 30 min post-tetracosactrin level was 17·3 μg/100 ml (±s.d. 6·2) compared with the mean response in normal adults of 31·4 μg/100 ml (±s.d. 5·2) recorded by Wood *et al.* (1965). Clearly there was evidence of significant H.P.A. axis suppression, and when the sixteen patients in whom the test was performed were examined separately, only four reached the normal response level.

This contrasts impressively with the children in the hydrocortisone treatment group where all except one, who had probably received topical steroids prior to admission, had a normal response to tetracosactrin prior to therapy. The 2nd post-treatment day tests gave completely normal responses in all of the nine children, the mean resting level of plasma cortisol being 14·5 μg/100 ml (±s.d. 7·5), rising to 41·3 μg/100 ml (±s.d. 18·2) after tetracosactrin.

DISCUSSION

The pattern of results in the adult cases is clear, namely those topical corticosteroid ointments which are therapeutically very efficient tend to be absorbed from the diseased skin and give suppression of the HPA axis. A clinical assessment of topical steroid enables them to be divided into groups of 'strong', 'medium' and 'weak' and as more preparations come on the market one can include other categories such as 'very strong' and 'very weak'.

Preparations such as Metosyn, Locoid, Betnovate and Locasalen would be considered in the 'strong' group and it is important to note that fluorination and 'strong' are not necessarily associated. Relatively 'weak' preparations include hydrocortisone and Locorten. Other investigations, not reported in this paper, suggest that Synalar Forte cream (fluocinolone acetonide 0·2%) and Dermovate ointment (clobetasol propionate 0·05%) should be considered as 'very strong' medications from both their clinical activity and their suppression ability.

The Betnovate ointment group of adult cases showed evidence of HPA axis suppression, with recovery of function occurring rapidly after treatment stopped, but to some extent this recovery of function was already starting while therapy continued, as the skin healed and the barrier zone in the stratum corneum regenerated.

When the stringent protocol of the investigation is considered, it becomes clear that even with intensive use, the dangers associated with HPA axis suppression must be minimal and this applies to all the corticosteroids tested. Betnovate does not differ greatly from Locoid in its suppression potential, and it has been demonstrated that significant though mild suppression does occur with Locoid (hydrocortisone butyrate 0·1% cream) using 60 g daily with polythene occlusion, so that claims made in advertising literature that the preparation is 'as safe as hydrocortisone' have not been substantiated.

The example shown in Fig. 2 of a patient treated with both Locorten ointment and later Betnovate ointment demonstrates the difference in the suppression action of these two compounds and in general terms this difference correlates well with clinical efficacy.

The topical corticosteroids which have previously been marketed do not show greatly differing effects in relationship to their clinical potency, HPA axis suppression and local atrophogenic action, but a dissociation between these features may be present in more recently produced steroids, and these factors must in future be exploited to maximum extent (Munro & Wilson, 1975).

Various accelerants such as dimethyl acetamide and dimethyl sulphoxide have been known to aid absorption of corticosteroids (Munro & Stoughton, 1965). In the present study the accelerant effect of adding 2% salicylic acid to Locorten ointment, or the use of polyethylene occlusion of the skin after application of Metosyn cream, has been shown by greatly increased absorption and HPA axis suppression when compared with plain Locorten ointment and with Metosyn cream covered only by stockinette.

The thin stratum corneum of the child predisposes to absorption of topical medications, and toxic effects from absorption of hexachlorophane and mercurial salts are well documented. Suppression of HPA axis function was present in twelve of the sixteen children on the 2nd post-treatment day following therapy with Betnovate ointment. The potential for long-term suppression by the prolonged use of Betnovate ointment in infants who have chronic eczema is clearly a risk. Growth impairment has been shown in an infant treated with Betnovate ointment for 3 years using 30 g of ointment each week (Munro, 1975) and it is encouraging therefore to see that the children treated with hydrocortisone ointment showed normal responses to tetracosactrin stimulation in all cases.

An additional point of interest in the children was the length of time required for the skin to be sufficiently clear of disease to stop treatment. In the Betnovate ointment cases the mean value was 12·9 days (±s.d. 6·3) and in the hydrocortisone ointment cases the comparable time was 15·7 days (±s.d. 4·1). This difference is quite small and much less than would have been suspected from general clinical impressions.

From all these studies it may be concluded that in adults, while the potential for suppression of the HPA axis exists when the most potent topical corticosteroid ointments are used, recovery of function occurs quickly when therapy is stopped. The benefits of the treatment appear to outweigh the systemic risks of the therapy.

In children the risk of chronic low-grade HPA axis suppression is greater, and whenever possible hydrocortisone ointment rather than the more potent preparations should be used. The benefit of the stronger medications should not be denied to these children, however, but the weakest preparation which will control the disease should be the ointment of choice.

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DISCUSSION

DR C.J.GUERRIER (NORTHAMPTON): May I ask if you have any data on the hydrocortisone preparations made in urea-containing vehicles?

DR D.D.MUNRO: I have not done any studies on this.

DR P.J.AUGUST (MANCHESTER): I should like to mention some of the data obtained at St Thomas's (Carruthers, August & Staughton, 1975). Using 12·5 g of Dermovate ointment on only half the body surface without polythene was enough to reduce serum cortisol over 4 or 5 days to 1 or 2 μg/100 ml in four of five subjects tested. Halving the dose to 6 g per day, caused no reduction in serum cortisol in any of the six subjects tested.

Reference

CARRUTHERS, J.A., AUGUST, P.J. & STAUGHTON, R.C.D. (1975) Observations on the systemic effect of topical clobetasol propionate (Dermovate). *British Medical Journal*, **iv**, 203.

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Measuring adrenal function in out-patients using topical corticosteroids

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SUMMARY

A number of studies describing the effects on pituitary adrenal function of topically applied corticosteroids are reviewed. It is concluded that the adrenal function of the majority of out-patients is little affected by treatment with correctly prescribed topical corticosteroids. However, where patients need brief periods of treatment with large quantities of potent topical corticosteroids for extensive disease, clinicians should be aware of the possibility of transient adrenal suppression.

It is now well documented that corticosteroids applied to the skin can be absorbed percutaneously and exert a systemic effect (Scoggins, 1962; Kirketerp, 1964; James, Munro & Feiwel, 1967; Keczkes *et al.*, 1967; Munro, 1976). The degree of absorption and the subsequent effects are dependent on a number of factors including the intrinsic activity of the corticosteroid, its formulation (Barrett *et al.*, 1965) and its mode of application. When examining the effects of a particular corticosteroid preparation on adrenal function it is necessary to assess the results of intensive use such as has been described by Munro (1976). However, in interpreting the clinical significance of the findings it is also helpful to monitor its effect in large numbers of unselected patients using the sort of quantities normally employed.

There are a number of methods of assessing the effect of corticosteroids on adrenal function (James & Landon, 1970; Landon, 1976). Some are complicated and are not always practicable for use in out-patients. The insulin stress test is the most informative single test available but it necessitates keeping the patient in hospital for the day and is therefore time-consuming and not easy to conduct in a busy out-patient clinic. The use of the short tetracosactrin test (James & Landon, 1968) is simple, but again is time-consuming, and involves patients staying in the clinic for longer than usual; it also requires careful timing of blood sampling and this is not always practicable when large numbers of patients are being examined. Collection of 24 h urine samples for estimation of oxogenic steroids is not easy for the out-patient. The most straightforward method is to take single blood samples for estimation of plasma cortisol levels. However, in interpreting the results a number of factors must be borne in mind.

It is known that plasma cortisol levels undergo significant circadian variation (James & Landon, 1970) and also that corticosteroids are released from the adrenals in bursts, and levels therefore also undergo episodic variation (Gwinup & Johnson, 1975). The circadian rhythm correlates with the sleep–wake cycle and, in the majority of patients, levels are at a peak at about 9.00 a.m. After this they decline and patients with normal adrenal function can have very low levels by late afternoon. Thus, if plasma cortisol levels are to be used to assess adrenal function, ideally a number of consecutive 9.00 a.m. estimations should be carried out, which again is normally an impractical proposition in an out-patient clinic. Further complications are added by the fact that stress can increase cortisol levels; out-patients are exposed to a number of stressful situations not least of which is the prospect of venepuncture and this has been shown to alter results (James & Landon, 1970; Zumoff *et al.*, 1974). There is also a suggestion of a sex difference, men having higher levels than women (Zumoff *et al.*, 1974). A seasonal variation has been demonstrated, levels being higher when the weather is either very hot or very cold (Watanabe, 1964). The plasma cortisol level of women taking oral contraceptives is markedly increased, frequently reaching those that are found in Cushing's syndrome. However, symptoms of this condition are not seen, presumably because the cortisol is not free but bound to transcortin (Lucis & Lucis, 1972). This phenomenon may also occur with other drugs.

In spite of these difficulties a number of studies have been carried out in out-patients, using plasma cortisol levels estimated from single blood samples as the method of assessing the effect of percutaneously absorbed steroids on adrenal function.

Out-patient studies

Wilson, Williams & Marsh (1973) studied 295 subjects who were being treated with topical steroids in the out-patient unit of a hospital. They compared them with 158 controls. All patients who visited the clinic and were using topical corticosteroids were included. Ninety per cent of the patients being treated were using betamethasone valerate preparations in quantities ranging from less than 15 g to 500 g per week. Assessment of adrenal function was solely by single estimates of plasma cortisol levels taken at either morning or afternoon out-patient sessions yet, in spite of this, only six of the treated

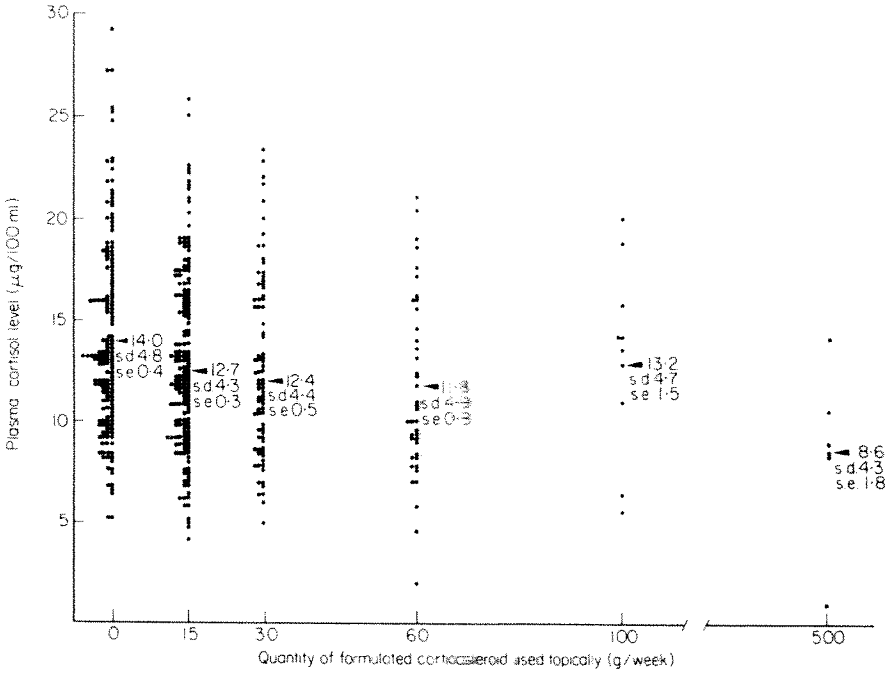

FIGURE 1. Plasma cortisol levels of 295 out-patients using various amounts of topical steroids compared with 158 controls (Wilson *et al.*, 1973). Arrow, mean level; s.d., standard deviation; s.e., standard error.

patients had levels less than 5 μg/100 ml. Thus even though up to 500 g per week was being used the majority of patients had normal levels (Fig. 1). At about the same time Munro & Clift (1973) studied a similar though very much smaller group of forty patients, thirty-one of whom had received therapy with topical corticosteroids for 3–6 years. In this study adrenal function was assessed by the insulin stress test and thirty-seven of the forty patients had a normal response on the first testing. The remaining three produced essentially normal results 2–5 months after using half their previous dose, which the authors thought excessive. The use of this test confirmed that the whole of the hypothalamic pituitary adrenal axis was unaffected.

Walker *et al.* (1974) carried out a study in which thirty-four patients who needed treatment with potent topical steroids had their adrenal function monitored while applying clobetasol propionate ointment. An initial measurement was made followed by measurements after 2 and 4 weeks' treatment. As with the Wilson *et al.*'s (1973) study, assessment was by estimation of plasma cortisol levels taken either at the morning or afternoon clinic. The results for the estimation at 2 weeks are shown in Fig. 2.

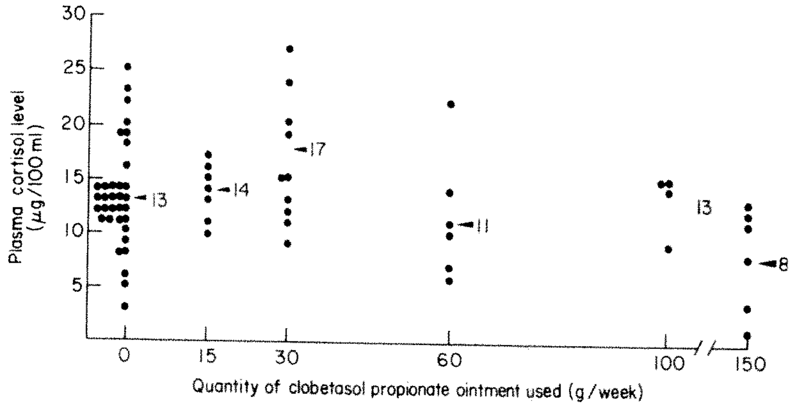

FIGURE 2. Plasma cortisol levels of out-patients using clobetasol propionate ointment (data from Walker *et al.*, 1974). Arrow, mean level.

Though the numbers involved were very small, the study showed that when used in reasonable quantities there was little effect on adrenal function. However, when large quantities of ointment were used, changes in cortisol levels were seen which tended to be dose related.

A series of thirty-nine subjects, using various quantities of clobetasol propionate ointment, fifteen of whom were out-patients and twenty-four in-patients, were studied by Allenby *et al.* (1975). Assessment of adrenal function was by the short tetracosactrin test at the beginning and end of treatment. With the in-patient group it was possible to perform regular 9.00 a.m. plasma cortisol estimations as well. Fig. 3a shows the plasma cortisol level taken prior to injection of tetracosactrin for out-patients using less or more than 50 g of ointment per week. The number of patients is small but a fall in plasma cortisol levels is seen with increasing use of the steroid preparation. The average length of treatment in this group was 17 days. Fig. 3b shows the findings from the in-patient group in which the average length of treatment was 14 days. If the levels at the beginning and end of treatment are considered, the same trend of falling plasma cortisol levels with increasing use of steroid is seen. However, as with these patients it was also possible to monitor daily 9.00 a.m. blood samples during treatment; the lowest plasma cortisol levels recorded could be observed and these are shown in Fig. 3b. They tended to be found shortly after the ointment was first applied and were lower than those found at the end of treatment. This is a similar finding to that of James *et al.* (1967) who noted that plasma cortisol levels

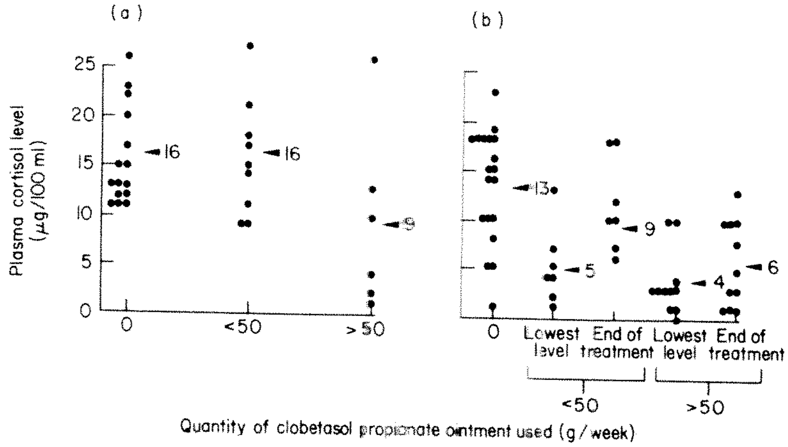

FIGURE 3. Plasma cortisol levels in (a) out-patients and (b) in-patients, using clobetasol propionate ointment (data from Allenby *et al.*, 1975). Arrow, mean level.

tended to increase during the last few days of therapy in two in-patients treated intensively with betamethasone valerate under polythene occlusion and is probably due to the restoration of the dermal barrier. Thus the sensitivity of the test as a measure of the effects of corticosteroids is greatly increased if assessment of adrenal function is made as soon as possible after the start of treatment.

Another study monitoring both in-patients and out-patients was that by Keczkes *et al.* (1967) in which triamcinolone acetonide ointment was used. The steroid was made up in differing strengths, patients receiving a progressively reducing dose. Plasma cortisol levels were not estimated in the out-patient group but a fall in urinary 11-hydroxycorticoids and cortisol secretion rates was found, indicating that even under a 'reducing dosage' regime an effect on adrenal function was detectable. However, they could not detect a significant relationship between the amount of triamcinolone acetonide applied and the degree of suppression.

The corticosteroids used in the investigations so far described have been those with potent anti-inflammatory activity. Until recently it has been thought that high topical activity is always accompanied by systemic effects if the quantity of steroid used is large and other conditions which predispose to percutaneous absorption obtain. However, clobetasone butyrate, although not as effective clinically as such steroids as clobetasol propionate, nevertheless has more topical activity than hydrocortisone, flurandrenolone and flucortolone and seems to show a greater degree of dissociation of systemic from topical potency than hitherto observed.

Intensive use of clobetasone butyrate as a 0·05% ointment with polythene occlusion has been shown to have minimal effects on adrenal function (Munro & Wilson, 1975). This steroid has also been tested in out-patients in trials designed along similar lines to that of Walker *et al.* (1974). In view of the finding that maximum lowering of plasma cortisol is likely to occur soon after the start of treatment, blood samples were taken when treatment began and after 7 days.

The results from two centres have been combined and are shown in Fig. 4. As might have been expected from the results of Munro (1976), in this instance there was no indication that increasing dosage produced any depression of plasma cortisol levels, even though quantities of up to 150 g per week were being used.

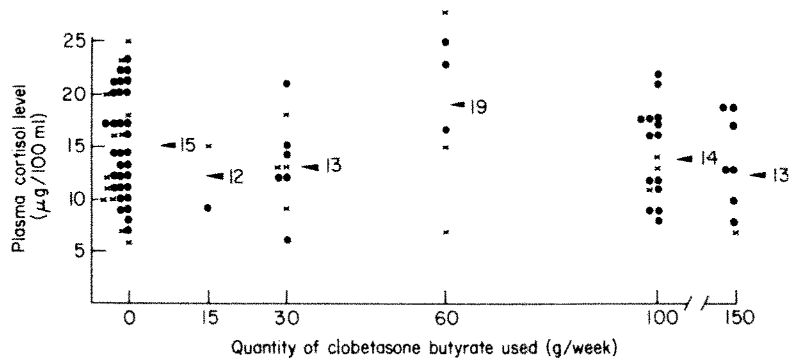

FIGURE 4. Plasma cortisol levels of out-patients using clobetasone butyrate ointment (●), or cream (×) (Bowyer, Sparkes, Fry & Walker, unpublished). Arrow, mean level.

DISCUSSION

These studies have demonstrated that in spite of the fact that assessment of plasma cortisol levels as a measure of adrenal function has its limitations it can be used to give an idea of the propensity of corticosteroid preparations to cause adrenal suppression. Further, this is more likely to be seen if the test is made as soon as possible after the start of treatment. It must be borne in mind that some patients who have a normal plasma cortisol level will respond abnormally to other tests of hypothalamic pituitary adrenal function. However, in the study by Allenby *et al.* (1975) only one patient who had normal plasma cortisol levels at the end of treatment failed to respond normally to tetracosactrin.

They also demonstrate that with some preparations it is possible to show lowering of plasma cortisol levels when large quantities are used. The degree of effect on adrenal function for a given quantity of steroid preparation applied varies between preparations. With preparations with activities of the order of clobetasone butyrate it is necessary to use the type of test described by Munro (1976) to establish its relative ability to effect adrenal function. This is because in out-patients it is not normal to use sufficiently large quantities of ointment to have a detectable effect. Where it is necessary to use potent steroid preparations in large quantities or where effects on adrenal function are particularly undesirable such as in children, information provided by the more severe test is useful. In view of the minimal effect clobetasone butyrate has in this stringent test it is hardly surprising that no effects were seen in out-patients.

The majority of the conditions for which topical corticosteroids are used tend to be chronic and to wax and wane in their intensity. It is likely that during very severe exacerbations, patients use more creams and ointments than when the disease is under reasonable control. It is during these phases of more liberal treatment that an effect on adrenal function is most likely to be seen. The subjects described were seen in the hospital out-patient clinics and presumably had an exacerbation of their disease. They were thus likely to be using more of the preparation than is usual and because of a disrupted epidermal barrier were likely to absorb more.

In a number of experimental situations it has been shown that the majority of corticosteroids if applied in sufficiently large quantities and in the right conditions will be absorbed sufficiently to have effects on adrenal function. However, the studies reviewed here have investigated the adrenal function of patients using the quantities of corticosteroid preparations that are normally prescribed in hospital out-patient clinics. The majority of these patients were using small quantities of ointments and creams and were using them without occlusion. This is demonstrated in the study by Wilson *et al.* (1973) where about 80% of the patients were using less than 30 g per week. Since the amounts reported here are likely to reflect those in general use it is probable that the adrenal function of the majority of patients is very little affected by treatment with topical corticosteroids. However, where large quantities of the more potent preparations are used adrenal suppression is likely to occur, especially early in treatment. As long as these large doses are not used for a prolonged period adrenal atrophy and its attendant complications are unlikely to occur. Occasionally case histories are reported where patients have used abnormally large quantities of potent steroid preparations for unusually long periods of time (Staughton & August, 1975). In these extreme circumstances Cushing's syndrome and adrenal insufficiency on withdrawal of the steroid preparation have been seen, but for patients who have these preparations properly prescribed, adrenal function should rarely be affected. Occasionally it will be necessary for patients with extensive disease to be treated with large quantities of potent preparations for a short time and in these conditions clinicians should be aware of the possibility of transient adrenal suppression.

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Corticosteroids and pustular psoriasis

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Pustular psoriasis is a term applied to a group of disorders thought to have certain clinical, histological and aetiological features in common. The central clinical feature is a sterile intra-epidermal pustulosis which may be fulminating and generalized, prostrating the patients, or conversely localized and indolent. The latter type is usually confined to the palms or soles or less commonly the tips of the digits. Patterns between these extremes occur and a simple clinical classification is given in Table 1.

TABLE 1. Classification of pustular psoriasis

Localized	Generalized
Chronic palmo-plantar pustulosis	Acute—von Zumbusch type
Acrodermatitis continua of Hallopeau	—exanthematic
Pustular bacterid	Subacute annular (Lapière)
	Chronic annular
	Localized psoriasis with pustules

All these forms of pustular psoriasis were described long before the steroid era, i.e. they are certainly not artefacts of modern therapy. Nevertheless, there is no doubt that steroid therapy, systemic and topical, has had an impact on pustular psoriasis whose possible components can be separated, and at the same time defined, in the following questions:

1. May steroid therapy cause non-pustular forms of psoriasis, e.g. psoriasis vulgaris or generalized exfoliative psoriasis, to become pustular?
2. May such therapy convert chronic localized pustular psoriasis into more severe generalized disease?
3. Do steroids perpetuate or modify the natural history of spontaneously occurring generalized pustular psoriasis (GPP)?

In each of these questions, systemic and topical therapy may have to be considered separately if the evidence warrants it. In addition, the impact of withdrawal of treatment as well as its exhibition may be relevant. Quite separately, the place of topical or systemic steroid in the current management of pustular psoriasis must be assessed.

This paper summarizes the author's experience of the above problems over more than a decade and draws heavily on a large series of patients studied with T. Ryan (Baker & Ryan, 1968; Ryan & Baker, 1969, 1971) and on personal cases since 1971.

Conversion of non-pustular psoriasis

Throughout the 1950s and 1960s, a steady trickle of isolated case reports fed the suspicion that steroid therapy could be implicated in conversion to GPP (Alt & Many, 1953; Prakken, 1957; Schuppener, 1958; Champion, 1959; Muller & Kitzmiller, 1962; Rimbaud, 1964; Temime, Privat & Benne, 1966). Baker & Ryan (1968), studying 104 cases of GPP, found that thirty-seven of these patients had received steroids systemically for various reasons before the onset of the first attack of GPP.

In twenty-one instances, pustulosis had supervened within a few days or weeks of reduction of steroid dosage or the complete withdrawal of the drug and a causal relationship seemed likely. In the sixteen others, the relationship was not obvious because of a longer time lag or evidence that the psoriasis was already deteriorating seriously before steroids were administered. Thus, 20% of the patients in this large series were thought to have steroid-provoked GPP.

Two noteworthy features of the apparently steroid-provoked cases were the previously banal, ordinary discoid pattern of the disease in the majority and its generally long prepustular duration (Baker & Ryan, 1968). These features were in striking contrast to those of other patients with atypical forms of psoriasis, often of late onset, which rapidly and apparently spontaneously evolved into GPP. These and other observations led the authors to postulate the existence of radically different spontaneous and iatrogenic types of GPP (Table 2) with the implication that the widespread clinical impression of an increased incidence of GPP during the 1960s was due to systemic steroid therapy of ordinary psoriasis. Further cases supporting these conclusions were published by Agache, Barale & Bonjean (1970).

TABLE 2. The two types of generalized pustular psoriasis

	Type I	Type II
Onset of first psoriatic manifestation	Early	Late
Pattern of pre-pustular psoriasis	Typical	Atypical
Length of pre-pustular phase	Long	Short
Generalized pustulation	Provoked	Spontaneous

Subsequently, Ryan & Baker (1971) extended their observations to a total of 155 cases of GPP, particularly in regard to prognosis of the disease. They reported that thirty-seven patients (24%) developed their first attack of GPP within a month of first receiving steroids or within a month of their withdrawal. There was a little evidence that two or more provocative factors may have had an additive or even synergistic effect as happened in a recently published case where the combination of steroids and hypocalcaemia was devastating (Risum, 1973).

It may be objected that these observations were biased by case selection, and argued that systemic steroid therapy was used only in severe and unusual cases and that the advent of GPP is related to this rather than to any therapy. Certainly, if it could be shown that 24% of all patients with psoriasis in the U.K. were treated with steroids systemically in the period concerned, the argument against steroids would collapse but this proposition is patently ridiculous. Furthermore, analysis of the clinical data showed that steroids were often used, not because of the particular severity of the psoriasis, but often arbitrarily, initiated sometimes by general practitioners, rather than by dermatologists (Baker, unpublished data).

The hazard of systemic steroid therapy in psoriasis must be kept in perspective. It is possible that not all psoriatics are prone to GPP however great the provocation and some are demonstrably resistant.

Case 1. A 39-year-old lawyer with long-standing mild discoid psoriasis developed pemphigus foliaceus which became almost universal and bullous after a long sea voyage during which his whole body was exposed to tropical sun. Oral prednisolone, 240 mg daily, was needed to bring the pemphigus under control and during this therapy, his psoriasis disappeared. Subsequently, the prednisolone dose was reduced exponentially to 10 mg over a few months, methotrexate being added when the dosage fell to 60 mg daily. As the dose of prednisolone was reduced from 25 to 15 mg the psoriasis returned, exactly in its previous pattern and extent, and did not deteriorate further when both the methotrexate and prednisolone were subsequently stopped completely, topical therapy sufficing to control the pemphigus foliaceus.

In another patient, the exhibition of oral triamcinolone unwittingly provided a therapeutic test which solved a difficult differential diagnosis.

Case 2. A 48-year-old male civil servant developed an erythroderma *de novo*. There was no previous or family history of psoriasis. Resistance of the condition to prolonged bed rest and topical therapy in hospital, the presence of a few guttate islands of unaffected skin and the gross keratoderma of the palms and soles led to suspicion of pityriasis rubra pilaris. Triamcinolone, 20 mg daily orally, failed to influence the erythroderma but when the triamcinolone was reduced, the patient promptly developed generalized pustular psoriasis of the von Zumbusch type, establishing the correct diagnosis. Methotrexate controlled the eruption and allowed total withdrawal of triamcinolone.

Can intensive topical therapy of non-pustular psoriasis cause GPP?

The answer would appear to be yes. Baker & Ryan (1968) described two patients in whom intensive whole-body occlusion with fluorinated steroid under polythene was followed by GPP. The author has seen four similar cases subsequently and several further cases following the use of clobetasol propionate (Dermovate) without occlusion were recently reported (Boxley, Dawber & Summerly, 1975; Carruthers, August & Staughton, 1975). Careful frequent observation of in-patient psoriatics treated topically with strong steroids reveals that transient and localized pustulation is not uncommon, during changes or breaks in treatment.

Can steroids convert localized pustular psoriasis to GPP?

There is no doubt that chronic localized pustular psoriasis of the palmo-plantar type can evolve into GPP but the event is a rare one. No reliable data exist on which to state the percentage risk but the author's estimate, based on his own experience, is that it is no greater than 1%. Furthermore, the evidence points to this generally not being a steroid-induced event. Of 170 cases of GPP (155 reported by Ryan & Baker (1971) plus fifteen further personal cases) 9% started as acrodermatitis continua of Hallopeau and 7% as palmo-plantar pustulosis. Of these twenty-seven patients, only two were apparently steroid-provoked, using the same criteria as applied earlier. In the majority the localized pustulosis was of short duration (less than 2 years).

Over the years, very many patients with intractable palmo-plantar pustulosis have certainly been treated with systemic steroids including triamcinolone (Robinson, 1966) albeit usually with small doses and the lack of reports of provocation of GPP suggests that most are resistant to this conversion. However, the report of Calkins, Reznick & Bauer (1957) leaves little doubt that induction of GPP is possible. They described a middle-aged man with a 40-year history of pustular psoriasis confined to the palms and soles. He was given prednisolone in large doses (up to 150 mg daily) for experimental reasons. Four days after abrupt withdrawal of the steroid he developed GPP.

Chronic palmo-plantar pustulosis may not be a homogeneous entity and recent HL-A studies did not show the same prevalence of HL-A 13 and HL-A17 antigens as in ordinary psoriasis (Svejgaard *et al.*, 1974). It would thus be of particular interest to study the HL-A constitution of those patients with chronic palmo-plantar pustulosis who have developed GPP or other manifestations of psoriasis.

Can steroids perpetuate psoriatic erythroderma and GPP?

In the author's view, the answer is unequivocally yes. Of the 170 patients referred to above, twelve had persistent GPP until steroids were withdrawn. Seven other patients had steroid-perpetuated erythroderma liable to pustular phases. Even methotrexate may fail to control the erythroderma until the steroid is withdrawn.

Case 3. This 56-year-old stockbroker's messenger had suffered from psoriasis and polyarthritis for many years. He was first seen by the author in November 1972, when he had generalized pustular psoriasis and active polyarthritis. He had been put on oral prednisolone 2 years earlier and the steroid had been continued, without a break, in doses up to 20 mg, despite which the psoriasis remained severe and sometimes generalized. He was Cushingoid and crippled. In recent months he had also been given methotrexate in small daily doses which had failed to influence the disease.

A larger single weekly dose of methotrexate (9 tablets) subsequently controlled his psoriasis and over the following months he was weaned off the prednisolone. As the dose of the steroid was lowered, his psoriasis steadily became more tractable. He is now on prednisolone 2 mg daily and yet needs only 5 mg weekly of methotrexate to keep his psoriasis under almost total control. The arthritis is in remission and he is working full-time.

Trends in use of steroids in psoriasis

In the U.K. and elsewhere, there is now a very widespread appreciation of the dangers of systemic or hyper-intensive topical steroid therapy in psoriasis. Although no national survey has been carried out since 1968, my impression is that the disease is getting less frequent than in the 1960s, and that the years since 1968 have seen a decline in the use of systemic and whole-body occlusion therapy, although less intensive use of topical steroids is as prevalent as ever.

Has systemic steroid therapy any role in treatment of GPP?

Where conservative treatment is of no avail and the severity of the disease or its complications compel active measures, methotrexate is the drug of first choice. If methotrexate is contra-indicated or causes toxicity or fails, systemic steroids may be unavoidable and may be life-saving in the short term. Later, the steroid should be withdrawn, and usually this can be achieved only when anti-metabolite drugs are simultaneously administered.

The preference for methotrexate over systemic steroid rests on four grounds; namely, superior therapeutic effect, absence of a perpetuating effect, lower mortality and lower morbidity directly attributable to drug action. Thus Ryan & Baker (1971), analysing thirty-four deaths in 155 cases of GPP, attributed nine directly to therapy (seven to steroids and two to methotrexate). Eight other patients who died from uncontrollable pustular psoriasis were on steroid therapy at the time of death.

Their paper failed to bring out the even greater differences in morbidity attributable to steroids or methotrexate, respectively. Almost every case treated with steroid suffered one or more (often multiple) forms of morbidity such as Cushingoid changes, gross striae, osteoporosis, candidiasis, etc. In contrast the morbidity attributable to methotrexate was quantitatively and qualitatively strikingly less.

Comment on the role of topical steroids in GPP rests even more insecurely on clinical impression rather than available data. The value of topical steroids seems to be inversely proportional to the severity of the pustulation. Methotrexate is relatively ineffective in low-grade Lapière forms of GPP which may be well controlled for long periods with topical steroids alone.

CONCLUSIONS

The advent of the steroid era has influenced the behaviour and management of psoriasis. Intensive steroid therapy, systemic or topical, has been added to the list of factors known to precipitate generalized pustular psoriasis from non-pustular forms of the disease. In contrast the generalization of chronic palmo-plantar pustulosis is a rare and usually spontaneous event, unrelated to steroid therapy, except in rare instances.

An increasing body of evidence casts doubt on the value of systemic steroid therapy in the medium and long-term management of generalized pustular psoriasis where methotrexate is probably more effective and less toxic.

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DISCUSSION

DR J.L.BURTON (BRISTOL): You mentioned one patient who had both steroid withdrawal and hypocalcaemia. Do you measure serum calcium routinely?

DR H.BAKER: We have had calcium data in most of the patients under our care. They are often normal or slightly low. Certainly the number of patients who can be helped by giving calcium is very small indeed.

DR J.L.VERBOV (LIVERPOOL): What is your view about ACTH for pustular psoriasis?

Dr H.Baker: So far as provocation is concerned, I include ACTH with systemic steroids.

Dr R.C.D.Staughton (London): In the four cases of generalized pustular psoriasis reported from St Thomas's, to which we can now add a fifth, the patients were grossly Cushingoid as a result of topical clobetasol propionate. In four out of the five cases generalized pustular psoriasis resulted from withdrawal of the Dermovate.

Dr D.Boxley (Stoke-on-Trent): We have one more patient admitted 10 days ago who had apparently become erythrodermatous coincidentally with the use of Dermovate. On the day of admission she had a few small rings of pustules developing in her psoriasis, and we changed the steroid to prednisolone by mouth and gave her one dose of methotrexate. She is already improving. There is no further pustulation and normal skin is appearing.

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The response of psoriasis to betamethasone valerate and clobetasol propionate

A 6-MONTH CONTROLLED STUDY

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SUMMARY

Twelve patients with symmetrical psoriasis were studied for a period of 6 months. Clobetasol propionate 0·05% ointment was applied to the lesions on one side and betamethasone valerate 0·1% ointment to those on the other side as a double-blind controlled trial of these two treatments.

All the patients showed a relative improvement on the side treated with clobetasol propionate. Clobetasol propionate-treated lesions tended to clear for longer than the corresponding betamethasone valerate treated lesions. The condition of five of the patients was better at the end of the study period than at the beginning.

The activity of topical clobetasol propionate was reported by Sparkes & Wilson (1974). They used the method described by Williams *et al.* (1964) in which the assessments, and therefore the comparisons between treatments, are made 7 days after the beginning of treatment. The question was raised whether the same effect would be maintained for a much longer period.

Walker *et al.* (1974) showed that the use of large amounts of clobetasol propionate ointment was capable of causing pituitary–adrenal suppression in adults. This complication has been noted after topical treatment with betamethasone valerate but only in groups of patients especially at risk such as young children (Feiwel, Munro & James, 1968), patients with impaired liver function (Burton *et al.*, 1974) and in adults when the ointment was applied under occlusion with polythene (James, Munro & Feiwel, 1967).

A small study was designed to compare the effects of clobetasol propionate 0·05% ointment with betamethasone valerate 0·1% ointment in the treatment of psoriasis over a period of 6 months, while monitoring the function of the pituitary–adrenal axis by repeated Synacthen tests.

MATERIAL AND METHODS

Patients were chosen who had bilateral lesions of psoriasis which was symmetrical in site and severity, involving at most 15% of the body surface. A full explanation of the plan was made to a number of such patients and only those who seemed willing to comply with the scheme for the full 6 months were admitted to the trial.

The method used was a modification of the trial plan of Williams *et al.* (1964). It is a randomized double-blind trial plan in which comparisons are made at each assessment between pairs of matched lesions, one on each side of the body. Each patient was given a supply of ointment for lesions on the right side of the body and a differently labelled supply for lesions on the left. Neither patients nor doctor knew which ointment was used on a particular side. The patients were instructed how to apply the ointments so as to minimize contamination of one side from the other, and were to apply the ointments twice daily without occlusion. A short Synacthen test was done on each patient at the start of the trial and repeated at 3 and 6 months. All the tests were done between 2 and 3 p.m. on out-patients.

The patients were seen weekly for 1 month and monthly for the remaining 5 months. At each visit both patient and doctor compared the right and left sides. A difference in appearance was noted as a preference for the ointment used on the better side. If a lesion became no longer palpable, even if there was some residual erythema, it was noted as a remission and the patient was asked to stop applying ointment to it.

RESULTS

Fourteen patients were admitted to the trial (9 men and 5 women of mean age 44 years ± 18·4). Twelve patients completed the 6 months' trial period, one dropped out after 1 month and another after 3 months. Synacthen tests were done on twelve of the fourteen patients, one of whom did not complete the full trial period.

Agreement between the assessments of patients and doctor was good so the findings are reported together.

All the patients expressed a preference in favour of clobetasol propionate. The proportion of patients who expressed a preference at each assessment visit is shown in Fig. 1. With one exception, noted after only 1 week's treatment and not sustained, every time a difference was observed between a symmetrical pair of lesions, this was in favour of clobetasol propionate. The probability that all twelve patients would express a preference in favour of one treatment if the treatments were equal in effect is very small ($P < 0·001$). Thus the preferences show a significant difference in favour of clobetasol propionate.

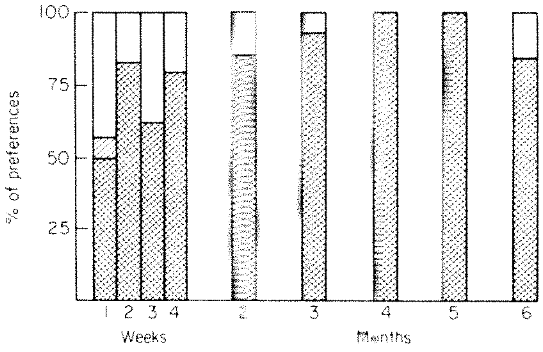

FIGURE 1. Comparative response of patients to clobetasol propionate and betamethasone valerate ointments at intervals throughout the trial. Open column, no preference; hatched column, preference for betamethasone valerate; cross-hatched column, preference for clobetasol propionate.

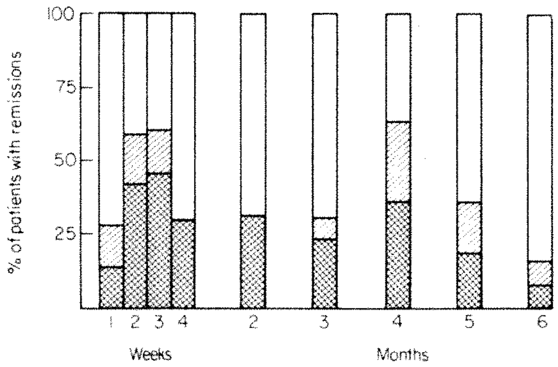

FIGURE 2. Comparative remission rates of lesions at intervals throughout the trial. Open column, patients not in remission; hatched column, remission of betamethasone valerate-treated lesion; cross-hatched column, remission of clobetasol propionate-treated lesion.

A remission was scored for a patient if one or more lesions on one side was in remission, thus Fig. 2 shows the proportion of patients who experienced partial or full remission of the lesions on one or both sides at each assessment time.

Four patients had no remission of lesions at all. Those patients who had a remission on both sides had a longer one on the clobetasol propionate-treated side than on the betamethasone valerate-treated side. There is an early peak when the clobetasol propionate-treated lesions were in remission for an average of 4 weeks and the betamethasone-treated lesions for an average of 2 weeks. Then several of the lesions tended to recur and treatment was continued on both sides. At about 4 months the second peak of remissions occurred. These were better sustained and averaged 10 weeks for clobetasol propionate and 9 weeks for betamethasone valerate.

The Synacthen test values are shown in Fig. 3 with the laboratory normal limits for Synacthen tests on out-patients. The normal limits comprise a band 2 standard deviations wide on either side of the mean. All the initial and final tests were within normal limits but in three of the tests done at 3 months though the initial plasma cortisol level was satisfactory the increment $\frac{1}{2}$ h after receiving Synacthen was low. The figure shows that the initial value means were all below the laboratory mean. This

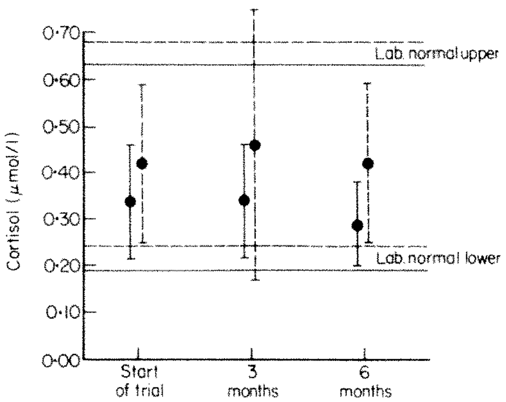

FIGURE 3. The response to short tetracosactrin tests before, in the middle and at the end of the trial, means and s.d. —— Initial values; – – – increments.

difference was tested by Student's '*t*'-test and was significant ($P<0{\cdot}05$) each time. This result was expected because the tests were all done during the afternoon while the normal limits are based on data from tests many of which were done during the morning. However, the increment means are not significantly different from the laboratory mean. Analyses of variance of both initial values and increments showed no significant difference between the groups of tests carried out at the three different times. None of the three low increments was less than 3 standard deviations below the laboratory normal mean, the patient had been using only small quantities of the trial ointments, and after careful consideration and discussion it was decided that these responses did not indicate a significant degree of pituitary–adrenal suppression.

The quantity of ointments used during the 6 months was similar: 60–360 g clobetasol propionate (mean 148 g), 90–360 g betamethasone valaerate (mean 145 g). The average used was less than 7 g of each ointment per week and the patient who used most used an average of only 15 g per week over the whole period.

One retrospective assessment was made for each patient at the end of the 6-month-study period. The psoriatic lesions of five patients were cleared or less marked than at the beginning of the trial, five showed no real change while two were worse on comparing the final state with the initial. In spite of the early encouraging results, by the end of the trial more than half the patients were no better than they had been at the beginning. Indeed two were worse and two others (one each from the improved and unchanged groups) had quite severe exacerbations soon after the end of the trial period.

DISCUSSION

Although the direct comparisons between pairs of lesions showed differences overwhelmingly in favour of clobetasol propionate, several of the lesions which cleared recurred again during the period of the study. The clobetasol propionate-treated lesions which cleared were all in remission longer than their opposite betamethasone valerate-treated lesions and, while no statistically significant difference in length of remissions could be shown, with such small numbers the power of this test to show any difference is quite low.

Relatively small quantities of the ointments were used and no pituitary–adrenal suppression was shown. Most of the patients treated their psoriasis less energetically towards the end of the trial period, possibly thus accounting for some of the decrease in contrast between the two sides which was noted towards the end of the study. However, it is clear that the preferences were achieved with the use of small amounts of ointment in marked contrast with the quantities used, for example, by the patients reported by Staughton & August (1975).

CONCLUSION

In this small study marked differences between the treatments in remission rates were not shown. There was, however, a strikingly unanimous preference for the clobetasol propionate ointment.

ACKNOWLEDGMENT

I would like to thank Dr H.T.H.Wilson for his help and encouragement.

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DISCUSSION

DR A.LYELL (GLASGOW): You did not have any experience of any pustules being provoked?

DR M.F.CORBETT: No, we used small quantities of ointment.

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The effect of corticosteroids on cell turnover in the psoriatic patient

A REVIEW

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Psoriasis is a disorder of epidermopoesis characterized by increased epidermal cell production with a rapid turnover time and shortened cell-transit time. It has been suggested that as a result of rapid transit the differentiating keratinocyte fails to mature, resulting in keratin that is macroscopically and microscopically abnormal.

In common with other treatments for psoriasis, topical steroids slow down this process by inhibiting epidermal cell division. This paper outlines the cell kinetics of the epidermal component of the psoriatic lesion and proceeds to show how cell behaviour is altered by treatment.

Turnover time

Turnover time is defined as the time taken for the epidermis to replace itself once and is the sum of the cell cycle time and differentiating time. Turnover time is equal to the average cell transit time from the basal layers via the prickle cell layers to the surface of the keratin layer.

Estimations of epidermal turnover time in psoriasis vary, possibly reflecting differences in techniques and between the patients. Weinstein & Frost (1968) considered the average turnover time for the epidermis beneath the keratin layer (EBK) to be only 2 days, whereas Goodwin, Hamilton & Fry (1974) calculated it to be 5 days which was in agreement with the calculation of Halprin (1972). If the turnover time for the keratin layer is an additional 2 days (Rothberg, Crounse & Lee, 1961; Weinstein & Van Scott, 1965) then the turnover time for the EBK plus the keratin layer is approximately 1 week, allowing for slight variations between patients.

Although precise information on the turnover time for normal epidermis is unavailable, most estimates suggest it is unlikely to be less than 4 weeks and it may be as long as 10 weeks (Halprin, 1972). Thus, the turnover time in psoriatic epidermis is probably 4–8 times more rapid than in normal epidermis.

Analysis of the epidermal compartment in psoriasis

The epidermis may be divided into three; the germinative, differentiating and keratin compartments. The stem, or germinative, cell population is involved in the cell-division cycle. Daughter cells from this compartment move outwards through the prickle cell layers that form the differentiating compartment, into the keratin layer.

Topical corticosteroids, like other drugs used to treat psoriasis, appear to slow down epidermopoiesis by inhibiting the cell cycle. They are acting either directly or indirectly on cells in the germinative cell population. In order to demonstrate these effects it is necessary to identify this group of cells. As there is no specific stain for them, recognition depends on cell morphology. It is possible to identify a group of cells in the basal layers that appear quite distinct from other cells higher in the epidermis. This distinction can be emphasized by Feulgen's DNA stain. These cells remain within the proximity of the basement membrane, are tightly packed, and have small dark staining nuclei with little surrounding cytoplasm. In contrast, cells in the higher layers have much more cytoplasm. Autoradiographs of psoriatic epidermis labelled with tritiated thymidine show labelled S phase cell nuclei distributed throughout these basal layers, suggesting that the cell cycle is confined to this zone. Further evidence to demonstrate the size of the germinative cell compartment is obtained from autoradiographs of psoriatic epidermis labelled with tritiated thymidine 24 h after methotrexate injection. For reasons not fully understood there is a large increase in the labelling index (the percentage of germinative cells labelled with tritiated thymidine) once again confined to this group of cells. It is noticeable that in many areas they are packed more than three cells deep which has previously been suggested to be the depth of the germinative cell layer.

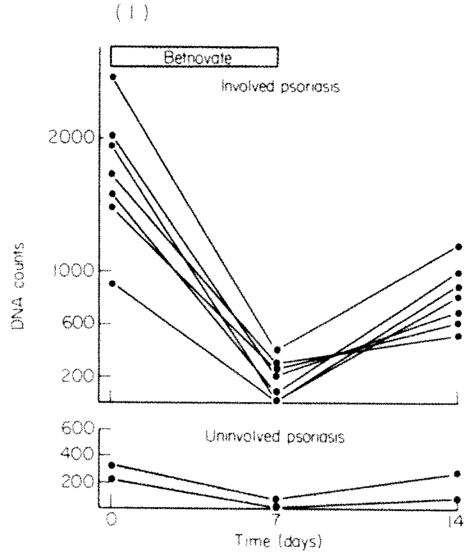
In their study of the cell cycle, Goodwin *et al.* (1974) concluded that out of a mean turnover time for the EBK of 120 h (5 days), 90 h were occupied by the cell cycle. Thus, approximately three out of four cells beneath the keratin layer were considered to be in the germinative cell compartment; this ratio is similar to that found by Weinstein & Frost (1968). In psoriasis the great increase in surface area of the basement membrane, with its exaggerated corrugations, is presumably to accommodate these germinative cells. From these calculations the keratinocyte appears to spend only 30 h undergoing differentiation, and this explains the failure to form mature keratin. At this point it is worth speculating that if the differentiating compartment expanded to compensate for the increase in size of the germinative cell compartment, more time and space would be available for differentiation. It is unlikely, therefore, that population pressure in the germinative cell compartment alone could account for the shortened maturation time.

Effects of topical corticosteroids on psoriatic epidermis

The striking histological feature of the epidermis after successful treatment with topical corticosteroids, apart from a reformed granular layer and the disappearance of parakeratosis, is the thinning and reduction in cellularity which, according to the criteria discussed earlier, appears to be due predominantly to a shrinkage in the germinative cell population. Van Scott & Reinertson (1959) suggested that drugs, including topical corticosteroids, which were effective in psoriasis, inhibited mitosis and thus slowed cell proliferation enough to allow maturation. In contrast, earlier studies by Komisaruk, Kosek & Schuster (1962) and McKenzie (1963) showed that the speed with which the granular layer began to return (12 h) suggested that these drugs acted on the outer layers of the differentiating compartment. Fry & McMinn (1968) confirmed that topical corticosteroids produce early changes in the granular layer and while it may be tempting to suggest a feedback mechanism between the end product (keratin) and the stem-cell compartment, they also reported a concurrent drop in mitosis inferring a direct anti-mitotic action.

Topical corticosteroids also suppress DNA synthesis in the epidermis. Goodwin & Fry (1976) showed that, after 1 week's treatment with topical betamethasone valerate ointment under polythene occlusive dressings, DNA synthesis was suppressed to levels similar to those found in the clinically uninvolved epidermis. In some patients DNA synthesis was totally suppressed. However, in all patients it rebounded after 1 week without treatment. In addition these authors have observed a similar effect in the clinically uninvolved epidermis in patients with psoriasis (Fig. 1). The implications of these results are discussed below.

Recent studies by Goodwin (unpublished data) have attempted to correlate the effects on the



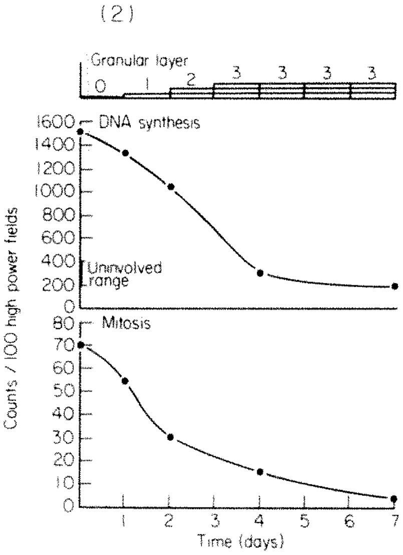


FIGURE 1. The effect of betamethasone valerate ointment, applied for 1 week under polythene occlusion, on DNA synthesis in the involved and uninvolved epidermis of patients with psoriasis. The epidermis was labelled with tritiated thymidine 1 h before biopsy, and from autoradiographs the number of labelled cells per 100 high power fields were counted. In some patients thymidine uptake was totally suppressed both in involved and uninvolved epidermis. In all patients the labelled cell count rebounded after treatment had been stopped.

FIGURE 2. The effect of topical corticosteroid under polythene occlusion on the granular layer, DNA synthesis and mitosis. Skin biopsies were taken 1 h after labelling with tritiated thymidine at 0, 24, 48, 96 h and 1 week after the beginning of treatment. DNA synthesis and mitosis were compared by counting the number of thymidine labelled cells and mitotic figures per 100 high power microscope fields. The granular layer was scored from 0 = absent to 3 = completely reformed.

granular layer, DNA synthesis and mitosis in the same patients. The results are shown in Fig. 2. Patients were treated with topical betamethasone valerate, under polythene occlusive dressings, and biopsies one hour after labelling with tritiated thymidine were taken over the course of 1 week.

Effects on granular layer

Reformation of the granular layer was rapid and in most patients complete within 2–4 days, although in common with other authors (Van Scott & Reinertson, 1959) we have noticed that it may be distinctly thickened. Fry, Almeyda & McMinn (1970) demonstrated granular layer changes in response to polythene occlusive dressings alone suggesting that other factors, such as hydration and temperature, contributed to this effect. Fry & McMinn (1966, 1968) also showed that the route of administration might affect the time sequence of these changes in relation to the anti-mitotic effect. Topically applied methotrexate (when it could be tolerated by the patient) reformed the granular layer before the mitotic count had dropped to normal. By contrast parenteral methotrexate produced a significant fall in mitosis before granular layer changes were seen.

Effects on DNA synthesis and mitosis

Results in Fig. 2 show a sharp drop in mitosis over the first 24 h which continues until 2 days after the start of treatment. Over this period the drop in DNA synthesis is less acute. Fig. 3 shows that

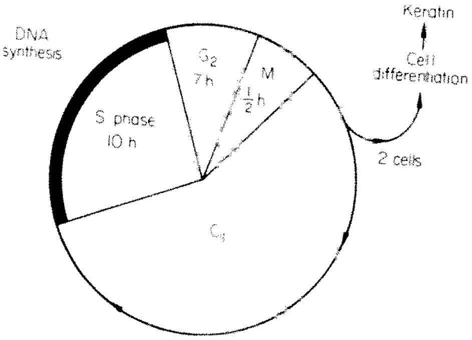


FIGURE 3. The phases of the cell cycle. After mitosis daughter cells may continue to cycle and enter G_1 or undergo differentiation. The duration of the phases is shown, according to the results of Goodwin *et al.* (1974); the total cycle time is believed to be approximately 90 h.

this is unlikely to be explained by suppression of DNA synthesis leading to a reduction in mitosis If $S\rightarrow G_2\rightarrow M$ takes 17 h, and allowance is made for absorption, such a steep fall suggests that topica corticosteroids are exerting a direct antimitotic effect (or alternatively blocking G_2).

However, after 4 days, DNA synthesis had fallen to within the levels found in clinically uninvolved epidermis whereas mitotic counts were dropping more slowly and were considerably higher than the range (1–2 per 100 high power fields) found in uninvolved epidermis. It is, therefore, possible that after 2 days, topical corticosteroids exert their anti-mitotic effect indirectly by inhibiting S-phase cells.

The clinically uninvolved epidermis

It was noted earlier that topical corticosteroids suppress uptake of tritiated thymidine by basal cells in the uninvolved epidermis. It has long been recognized that the uninvolved skin is abnormal in patients with psoriasis. Hell & Hodgson (1966) showed that after tritiated thymidine the labelling index was increased. Goodwin, Hamilton & Fry (1973) confirmed this and noticed that mitosis was also increased. Hodgson & Hell (1975) have subsequently demonstrated that in the eruptive phase, thymidine uptake by cells in the uninvolved epidermis can be stimulated by the injection of ground skin extracts, suggesting an increased reactivity as can be demonstrated clinically by the isomorphic response. Goodwin *et al.* (1973) have drawn attention to the large ratio between labelled epidermal cells and mitoses (>200 : 1). Duration of mitosis is unlikely to be less than $\frac{1}{2}$ h, as found in involved epidermis, and may be longer. S-phase duration has recently been shown by Goodwin *et al.* (unpublished data) to be approximately 12 h. In contrast to the cell cycle in involved epidermis, where it is believed that labelled cells pass from $S\rightarrow G_2\rightarrow M$ without delay, the majority of labelled cells in uninvolved epidermis are not preparing for cell division. It is suggested that these labelled cells take up the labelled DNA precursor as part of a DNA repair or other metabolic process.

It has been mentioned earlier that topical steroids applied to uninvolved skin reduce the number of labelled cells. In some patients labelling disappeared altogether which by inference suggests that not only is S phase suppressed, but also DNA repair, or whatever the mechanism is that causes uptake of labelled thymidine by this other group of cells.

DISCUSSION

Various effects of topical corticosteroids on cell behaviour in the epidermis of patients with psoriasis have been described. These effects may eventually be correlated. It seems that epidermal turnover is slowed by dual effects at separate stages of the cell cycle. The anti-mitotic effect is similar to the effect of colcemid but, unlike this drug, corticosteroids also block DNA synthesis and thus prevent an accumulation of cells in earlier phases of the cycle.

If DNA synthesis and DNA repair occur in uninvolved epidermis it is interesting that both these mechanisms are suppressed by corticosteroids, although hardly surprising, as it is possible that similar synthetic pathways are involved.

The relationship between granular and outer layers and the germinative cell compartment is still unexplained. A feedback mechanism from the keratin to the cells in the cycle is an attractive theory, but has several flaws: the early sharp fall in mitosis probably occurs within too short a time for a chemical message to diffuse back; also studies on the effects of methotrexate show that the sequence of histological events can be reversed by changing the route of administration.

Clinical experience with topical corticosteroids has shown that their anti-psoriatic effectiveness is related to potency as measured by vasoconstrictor assay (Sparkes & Wilson, 1974; Munro & Wilson, 1975). Goodwin & Fry (1976) showed that in some patients clobetasol propionate (Dermovate) produced greater suppression of DNA synthesis than did betamethasone valerate (Betnovate) with slower rebound after treatment was stopped, an effect that was reflected clinically as more rapid healing of the lesion with a longer interval of remission.

In conclusion, topical corticosteroids appear to have several actions, including inhibition of mitosis, suppression of DNA synthesis and possibly DNA repair and an ability to reform the granular layer. These observations suggest that the topical corticosteroids do not effect only one stage in the cell cycle.

The increased effect of a more potent corticosteroid such as clobetasol propionate has to be weighed against the concomitant increase in the collagen atrophogenic effects. It would appear prudent to reserve topical corticosteroids for use in patients when they can promote the natural tendency of psoriasis to remit. Courses of treatment with topical corticosteroids should be short, and continued use in those patients in whom the disease rebounds is inappropriate. The ideal topical drug would combine the efficiency of methotrexate with the sustained effect of dithranol and be cosmetically acceptable. Halogenated topical steroids are certainly effective and cosmetically acceptable and if there were better understanding of their action within the cell we might be nearer to formulating the ideal drug that separated the atrophogenic side-effects from the desired therapeutic effect.

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DISCUSSION

DR A.LYELL (GLASGOW): You have not paid any attention, have you, to that area between the involved and the uninvolved skin, that pale ring around the edge of psoriasis lesions?

DR P.GOODWIN: No, I have not. Presumably there is an interface, and one finds quite varied and unpredictable changes.

DR T.W.E.ROBINSON (LONDON): Can you be sure when you suggest that you were seeing the effect on mitosis you were not looking at the effect on G_2?

DR P.GOODWIN: No, we cannot be certain. It could be an effect on G_2, but as we do not know what G_2 is, it is something we cannot determine.

DR M.FEIWEL (LONDON): We have a fairly reasonable explanation of the action of methotrexate by the competitive inhibition of the relevant enzyme; I wonder whether you could speculate on the potential site of action of corticosteroids.

DR P.GOODWIN: I cannot, because I do not think any of us know sufficient about the biochemistry of synthesis within the cell.

DR E.S.SNELL (LONDON): One mode of action of steroids which might be relevant has been looked at. In the lymphocyte, glucose uptake of the cell is depressed and toxic fatty acids accumulate; there is some evidence that depression of DNA synthesis is mediated by fatty-acid accumulation.

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Clobetasol propionate ointment compared with dithranol in Lassar's paste in the treatment of psoriasis

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SUMMARY

The initial results on ten patients in a trial of clobetasol propionate (Dermovate) ointment compared with dithranol in Lassar's paste are described. The rates of clearing for each preparation in each patient were not noticeably different. Relapse was slower with clobetasol propionate in all but one patient for whom there was no preference. The results are highly significant ($P<0{\cdot}005$).

Psoriasis is a common chronic skin disease. At St Bartholomew's Hospital it accounted for 8·6% of new referrals to the out-patient's department in the first 6 months of 1975 as compared with 19% for warts, 15% for eczema, 4% for urticaria and 3% for all skin tumours. It is a condition characterized by long relapses and spontaneous remissions and accounts for a much higher proportion of out-patient follow-ups.

Such chronic conditions are the subject of numerous and diverse treatments which underline the lack of a cure or a perfect system of management. This is certainly true of psoriasis for which numerous topical and several systemic antimitotic agents are currently in use.

Cell poisons such as arsenic, mercury, resorcin and chrysarobin not only work in theory but have been used successfully in practice (MacKenna, 1959); similarly superficial radiotherapy is useful. However, their side-effects outweigh their usefulness. Current local regimens include ultraviolet light, topical dithranol, salicylic acid, coal tar fractions and the more potent steroids, while systemic regimens employ azathioprine, methotrexate and more recently azarabine (Keefer, Roenigk & Hawk, 1975) and, more rarely, systemic corticosteroids such as triamcinolone.

All but a small minority of patients respond promptly over 10–21 days to intensive in-patient treatment with coal tar baths, minimal erythema doses of ultraviolet light and twice daily applications of 0·05–2·5% dithranol in Lassar's paste. Modifications of this type of regime have been described by Ingram (1954) and Seville (1966).

The messiness of coal tar preparations and the staining and irritation of dithranol preparations with damage to clothes and the time necessary in executing therapy are often a deterrent to their effective out-patient use. When the staining and irritating properties of dithranol are modified and lessened a large part of the therapeutic efficacy is lost (Hellier & Whitefield, 1967; Hodgson & Hell, 1970).

The availability of topical halogenated steroids, for example triamcinolone, was welcomed as a major therapeutic advance. Later modifications such as the addition of water-soluble vitamin A were noted to increase their efficacy still further (Jarrett & Spearman, 1965). Odourless non-staining preparations rapidly cleared psoriatic lesions. Subsequently fluocinolone acetonide and betamethasone valerate became available topically but they brought about suppression of psoriatic lesions rather than permanent resolution. When therapy with these agents is stopped rapid relapse occurs while prolonged application leads to skin atrophy, telangiectasia and also to the skin lesions becoming refractory to further treatment.

Studies of the comparative effectiveness of topical corticosteroids and dithranol have given conflicting results. Knudsen (1965) noted that using fluocinolone, relapse occurred more rapidly than with conventional dithranol treatment, whereas Juhlin (1968) and Suurmond (1968) found evidence to the contrary.

The introduction of clobetasol propionate in a strength of 0·05% in the treatment of psoriasis suggested more rapid clearing of psoriatic lesions. In a double-blind study it was shown to be superior to other topical steroids in the treatment of psoriasis (Sparkes & Wilson, 1974).

Munro & Pringle (1975) have demonstrated, in a small group of cases, that clobetasol propionate ointment with or without added dithranol is as effective as is Lassar's paste with dithranol, and patients so treated tended to have longer periods of remission. The present trial was carried out to establish whether intermittent treatment with clobetasol propionate ointment is superior to conventional dithranol in Lassar's paste in the treatment of psoriasis.

MATERIALS AND METHODS

Patients with troublesome untreated symmetrical plaque psoriasis were admitted to the ward for intensive treatment in the trial. The preparations used were clobetasol propionate 0·05% in an ointment base (Dermovate) and 0·25% dithranol in zinc and salicylic acid paste B.P. (Lassar's paste) prepared in the St Bartholomew's Hospital pharmacy. A lower concentration of dithranol was used in some patients in whom the higher strength caused irritation.

Having been admitted the patients were examined and the type and distribution of their psoriasis recorded. The nature of the trial and of the ointments involved was explained to each patient before the trial. Treatment with twice daily applications by nurses of clobetasol propionate ointment to the lesions on the right side of the body and dithranol paste to the lesions on the left was begun on day 1 (Fig. 1). Scalp psoriasis was excluded from the trial and treatment. The treated skin was examined daily and the day of complete clearing of all lesions noted and recorded for each side.

On discharge, patients were instructed to use no local treatment but to make a careful note of the day on which psoriasis recurred on each side. The first follow-up appointment was 3 weeks after leaving hospital. The length of remission was taken as an index of the efficacy of each drug.

RESULTS

So far seventeen patients have entered the trial. Two have been lost to follow-up. In one patient, a 16-year-old boy with total body psoriasis, treatment had to be discontinued after 8 days when he entered a pustular phase requiring methotrexate.

The results of the first ten completed patients are recorded in Tables 1 and 2. All patients required between 10 and 21 days to clear their psoriasis and in only one patient, V.L., was there noticeable difference in the rate of clearing of the two sides.

Apart from patient C.C., in whom the psoriasis recurred simultaneously on the two sides, the skin

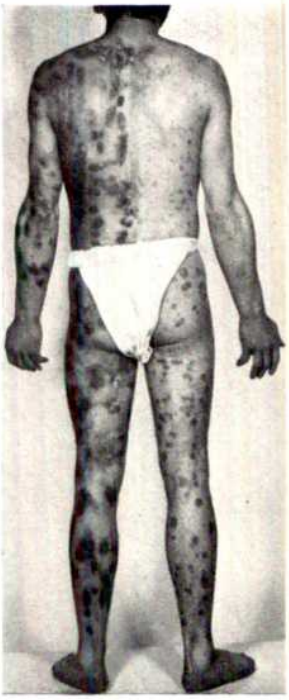

FIGURE 1. Patient treated with Dermovate ointment on right side and dithranol paste on his left.

TABLE 1. Length of treatment until clearance of all lesions (in days) for the first ten patients

	Time for clearance (days)	
Patient	Clobetasol	Dithranol
C.C.	14	14
J.D.	11	11
P.F.	18	18
R.J.	14	14
D.N.	10	10
V.L.	21	18
B.M.	16	16
L.O.	15	15
K.S.	14	14
F.W.	17	17

TABLE 2. Length of remission (in days) and treatment preference in first ten patients

Patient	Days clear after discharge		Preference	
	Clobetasol	Dithranol	Clobetasol	Dithranol
C.C.	11	11	0	0
J.D.	12	5	+	—
P.F.	8	1	+	—
R.J.	21	7	+	—
D.N.	18	15	+	—
V.L.	48	43	+	—
B.M.	22	14	+	—
L.O.	45	14	+	—
K.S.	14	7	+	—
F.W.	34	13	+	—

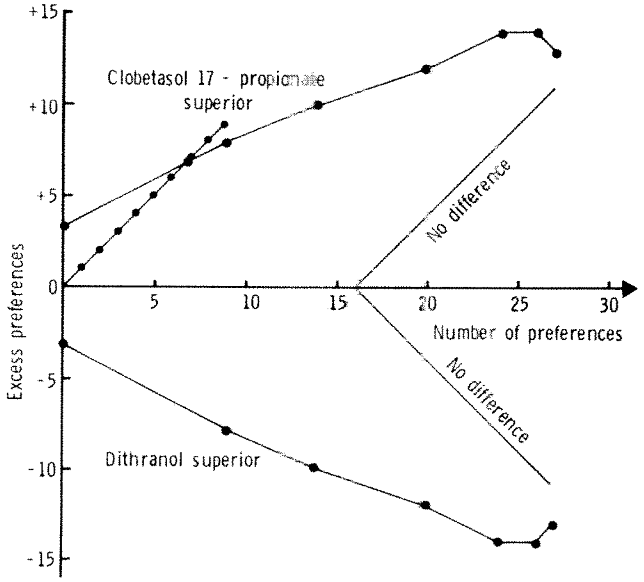

FIGURE 2. Sequential analysis showing seven consecutive preferences for Dermovate-treated side. Restricted sequential analysis $O = 0{\cdot}85$; $2\alpha = 0{\cdot}05$; $1-\beta = 0{\cdot}95$.

remained clear longer on the clobetasol-treated side in all patients (mean 23·3 days, ±s.d. 14·1) compared to the dithranol-treated side (mean 13·0 days, ±s.d. 11·5).

Armitage's (1960) method of restricted sequential analysis lends itself especially for use in trials involving small numbers of patients. The method ignores those who react equally ('tied pairs'). A design corresponding to a 5% level of significance was chosen (Fig. 2) and the clobetasol propionate boundary was reached when the seventh patient had completed the trial.

An alternative method of analysis described by Siegel (1956) does not employ sequential recording. Using this 'sign test' the hypothesis that there is no difference between the two preparations can be rejected in favour of the alternative hypothesis that there is a difference at the 0·5% significance level. In this investigation all preferences for the length of remission are for clobetasol propionate ($P < 0{\cdot}005$).

DISCUSSION

Psoriasis is a disease of remission and relapse. The course varies from patient to patient and at different times in the same patient. The advantage of the method used in this trial is that two treatments can be compared simultaneously. The very nature of dithranol, its staining and irritating action on the skin and its preparation in paste, precludes the use of a double-blind trial, when comparing it with clobetasol propionate ointment.

Table 1 suggests that clobetasol propionate ointment and dithranol in Lassar's paste produce equally satisfactory clearance of psoriasis over a similar time course. However, Table 2 shows a wide variation in the periods of remission induced by each preparation.

The mean remission rates have wide standard deviations. This may well reflect the nature of the disease in individual patients. With such a variation, selection of a patient with a remission differing greatly from the others could distort the mean and invalidate any conclusion drawn. By observing the behaviour of a patient's skin to the simultaneous use of two treatments establishing a 'preference' for one or other treatment and by the use of appropriate methods of statistical analysis these difficulties can be overcome.

Although at the beginning of this trial we selected 0·25% concentration of dithranol some patients experienced irritation. The concentration was reduced stepwise until this effect was overcome. We do not feel this invalidates the trial but rather that some patients who are more sensitive to dithranol react and respond at a lower dose. It could be argued that the right side of the body behaves intrinsically differently from the left. The trial continues at present with the sides of application reversed.

Although the present study is small the result is highly significant. We have shown clobetasol propionate to be superior to dithranol. It is preferred by patients for its cosmetic properties, absence of irritation and staining. If used intermittently in this way and with proper supervision, the atrophic effects seen with continuous therapy should be less. Thus it may have an important part to play in out-patient management of psoriatic patients in the future.

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DISCUSSION

DR G.HOLTI (NEWCASTLE): Topical applications are the only drugs in which the dosage is not specified by the prescribing doctor and more attention will have to be paid to this in the future. I find it perfectly acceptable to use potent corticosteroids such as Dermovate provided they clear an attack of psoriasis completely within 3 weeks, and are not used for a longer period.

DR P.J.MARRIOTT: I agree. If I am treating patients in out-patients with Dermovate, I always like to see them every fortnight and not every 3 months or so.

DR A.B.SHRANK (SHREWSBURY): I think it falls on the pharmaceutical companies to make it clear to the general practitioner and to the patient what sort of quantities are required for lesions.

DR E.S.SNELL (LONDON): The pharmaceutical companies have far less control over doses for patients than the doctor. It is the doctor prescribing it who has the greatest control over the dosage the patient receives. Dermovate is a steroid which has side effects and has to be used very carefully. The best source of information on this is the Data Sheet which we supply.

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Experimentally induced steroid atrophy in the domestic pig and man

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SUMMARY

A histological method of ranking topical steroid preparations according to the degree of epidermal thinning they cause in pig skin has been shown to be applicable to human skin. A 0·05% ointment of the new compound, clobetasone butyrate (Molivate), has been shown to cause less thinning than either fluocinolone acetonide 0·025% (Synalar) or flurandrenolone 0·0125% (Haelan) when applied without the use of occlusive dressings for 3 weeks to the human forearm.

A method of inducing and measuring epidermal thinning in the domestic pig by the topical application of glucocorticoids has been described by Winter & Wilson (1976a). Biopsies are taken before and at intervals throughout treatment and the relative effect of various steroids on epidermal thickness can be judged qualitatively by histological appearance and quantitatively by projecting an image of a cross-section on paper and measuring the area of the epidermis with a planimeter. A previous study in the pig using this technique (Wilson & Winter, 1976b) has compared clobetasone butyrate 0·05% (Molivate) with five other topically active corticosteroid ointments, flurandrenolone 0·05% (Haelan X), fluocinolone acetonide 0·025% (Synalar), triamcinolone acetonide 0·1% (Adcortyl), flurandrenolone 0·0125% (Haelan) and hydrocortisone 1%. It was found that the clobetasone butyrate ointment, applied under occlusion for 7 weeks, produced less epidermal thinning than any of the other active preparations except hydrocortisone.

Pig skin is anatomically similar to human skin, and a small preliminary study (Winter & Wilson, 1976a) in two human volunteers showed that the daily application of 0·025% fluocinolone acetonide under occlusion to forearm skin for only 7 days produced loss of the granular layer with thinning of the epidermis and disappearance of the epidermal ridges and dermal papillae.

The present study has compared the effects of three different steroid preparations applied to normal unoccluded human skin.

METHODS

We studied fifteen male patients aged 44–66 years, who were attending hospital for the treatment of localized dermatoses which did not affect the arms, such as seborrhoeic warts or basal cell carcinomas. The subjects were informed volunteers and the project was approved by the Hospital Ethical Committee. In fourteen subjects the flexor aspect of one forearm was treated with 0·025% fluocinolone acetonide twice daily for 3 weeks and that of the opposite forearm was treated with either flurandrenolone 0·0125%, clobetasone butyrate 0·05% or nothing. The fluocinolone acetonide was applied to the right and left arms in alternate patients in each of the three groups in order to cancel out any observed differences on the two sides which may be attributable to increased use of one arm, ease of application of ointment, etc. In one subject clobetasone was applied to one forearm, and the opposite forearm was untreated. After 21 days' treatment a 1 cm skin biopsy was taken from corresponding areas on each forearm and fixed in 10% formol saline. The skin specimens supplied for histology were coded and the identity of the treatment was not disclosed to the histologist (G.W.) until the qualitative and quantitative results had been determined. Serial sections 10 μm thick were prepared and stained with haematoxylin and eosin, and by the Weigert and van Gieson, and Masson's trichrome techniques. Qualitative assessment of the section was performed, and then images of the epidermis in cross-section were projected at × 150 magnification and the outlines traced. This was done for five different sections of epidermis, each 2·0 mm long, from each specimen of skin, and the projected cross-sectional areas were measured with a planimeter. The average thickness of the epidermis was calculated from the expression:

$$\text{Average thickness} = \frac{\text{total projected area}}{\text{total length examined} \times 150}.$$

RESULTS

Microscopic examination of the skin specimens treated with fluocinolone acetonide showed a number of epidermal features which we have come to recognize as typical of the effects of topical glucocorticoid treatment of skin. These effects are: thinning of the epidermis, loss of the granular layer, flattening of the epidermo–dermal border, pyknotic nuclei in the basal layer of the epidermis and a tendency for clusters of epidermal cells to appear above the normal plane of the granular layer. There was, however, marked variation in the appearance of the untreated skin in different patients and in their responses to treatment. The mean epidermal thickness of the untreated specimens varied from 28 to 56 μm, and that of the specimens treated with fluocinolone acetonide varied from 15 to 37 μm. The mean epidermal thickness for each group of specimens is shown in Table 1.

TABLE 1. Thickness (μm) of epidermis after twice daily treatment (without occlusion) for 3 weeks (mean ± s.e.)

Treatment	No. of specimens	Mean	s.e.
None	6	42	4·9
Clobetasone butyrate (Molivate)	6	37	4·3
Fluocinolone acetonide (Synalar)	14	31	2·7
Flurandrenolone (Haelan)	4	29	3·7

In the five patients in whom fluocinolone acetonide was compared with no treatment, the epidermis was in every case thicker on the untreated side, and the mean difference between the two arms (8·4 μm ± s.e. 2·82) was significantly greater than zero ($t = 2{\cdot}88$, $P < 0{\cdot}05$).

COMMENT

This study confirms that the histological technique previously applied to pig skin can also be applied to human skin to rank steroids in order of potency with regard to their potential to produce epidermal atrophy. Although the numbers are still too small for confidence limits to be applied, our results suggest that in man, clobetasone butyrate 0·05% produces considerably less epidermal atrophy than either fluocinolone acetonide 0·025% or flurandrenolone 0·0125%.

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DISCUSSION

DR J.E.WHITE (POOLE): How much of the epidermal changes are due to dermal changes in, say, the water content of the dermis or the mucopolysaccharide content of the dermis?

DR J.L.BURTON: I think the whole question of the relationship between dermal atrophy and epidermal atrophy is very difficult. We imagine that the two will go together and are bound to influence each other to a certain extent. But they may be entirely separate mechanisms.

DR A.JARRETT (LONDON): I think it is very well established that steroids are in fact strongly anti-mitotic, and I gather from what you say that atrophy is a bad thing. But it is not necessarily so. For instance, if you are treating psoriasis is it not a good thing to thin the epidermis a little?

DR J.L.BURTON: We are all hoping that eventually a whole range of compounds will be produced having subtle differences. We can then choose our compound according to the age of the patient, the type of skin and the particular disease we are treating.

MISS LYN WILSON (LONDON): Dr Goodwin showed that when you apply steroids to psoriatic skin, the first change that happens is that the granular layer reforms. In these experiments when one puts on steroids, the granular layer seems to disappear, and that seems to be fascinating.

DR J.L.BURTON. It is not surprising you get different effects in psoriatic skin which is very abnormal compared with what I hope is normally responding skin. I think that the pharmacological response often varies according to the disease being treated; in cardiac failure for instance, a drug will do one thing to the failing heart and something else to the normal heart. I think these small differences are well worth pursuing because of the insight they give into the pathogenic process.

DR P.G.GOODWIN (LONDON). I think what you showed are the same changes we see when we treat psoriasis, namely, a shortening and thus a reduction in the surface area of basal membrane with accompanying reduction in size of the basal cell population, presumably as a result of the suppression of DNA synthesis and mitosis.

DR D.F.BARRETT (PORTSMOUTH): Have you any information on how long it takes for the epidermal thinning to recover?

DR J.L.BURTON: It takes a long time in the pig's skin—about 12 weeks. We have not done any further biopsies in the human patient.

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Steroid-induced atrophy in an animal and human model

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SUMMARY

A ratchet-controlled micrometer screw gauge has been used to measure the changes in skin thickness produced by topical corticosteroids.

Measurable thinning of the mouse ear followed repeated daily application of corticosteroids. This response in the animal was used to assess the atrophic potential in a variety of standard formulations. The same compounds were applied to human skin and the changes in thickness measured.

Patterns of response and the rank order of thinning obtained from the animal model were found to parallel closely the human results.

Atrophic skin changes have long been recognized in association with adrenocortical hormone excess (Albright, Parsons and Bloomberg 1941; Behrman & Goodman, 1950).

Similar changes follow the topical application of corticosteroids. These compounds had been in clinical use for many years before this effect was noticed (Epstein, Epstein & Epstein, 1963; Sneddon 1968). The earlier reports described atrophy occurring after months or years of regular use. We now recognize that atrophic change can occur rapidly and that many factors influence this, e.g. body site, potency of steroid, occlusive effect, age, etc.

Many areas of the skin will recover months after stopping treatment (Epstein *et al.*, 1963). In others the faded marks of striae may remain. The rebound phenomenon (Sneddon, 1969) is still a problem in a small number of patients. In this same group of patients substitution of a less-potent steroid may be difficult. The problem is magnified when clinicians are unsure of the skin-thinning potential of a new corticosteroid. Misleading and flimsy evidence, provided by some manufacturers to allay our fears, complicates the problem. For these reasons it is essential to examine the skin-thinning properties of a topical corticosteroid before it is widely used in clinical practice.

Gross alteration in topical efficacy can be produced in a steroid compound by minor alterations to its vehicle.

Manufacturers choose an optimal base for each steroid, and it is their combined effect that we see in our patients. Throughout all experiments, commercially available topical medications have been applied. The use of trade names is deliberate for we are not investigating actual steroid chemicals only their effect in combination with their standard base.

Animal-laboratory workers have produced skin thinning in rodents by systemic use of ACTH or corticosteroids (Moon, 1937; Baker *et al.*, 1948). Topical application has effected similar change in rodent skin (Baker & Whitaker, 1948; Castor & Baker, 1950). It is unlikely therefore that rodent skin behaves in a totally different way from the human when treated with topical corticosteroids; but anatomical differences such as the increased number of skin appendages and the absence of a papillary dermis clearly influence absorption.

It is much easier to apply topical compounds on a hairless animal or to a relatively hairless part. The mouse ear was chosen as a convenient site to measure thickness change. Initially the hairless mouse was used in these experiments but the cost, housing, wasting disease and susceptibility to chilling in an open laboratory, provided sufficient reasons to examine the relatively hairless ear of the ordinary laboratory mouse.

Results comparable to those obtained from the hairless-mouse ear were reproduced in a common albino strain.

MATERIALS AND METHODS

Animal study. Male albino mice of the CDI Strain Charles Rivers U.K. Ltd were used throughout. Ten animals made up a group, all weighed around 40 g and were 5 months old.

Each mouse, housed separately, received applications of corticosteroids to its left ear. The right ear remained untreated throughout. All animals were anaesthetized with ether for measurement and the application of ointment. Whenever possible ointments rather than creams were used, and these were the manufacturers' standard preparations as used in clinical practice.

Tuberculin 1 ml syringes preloaded with the corticosteroid ointment under investigation delivered 0·01 ml onto the left ear and this was gently rubbed in.

On Wood's light examination it was demonstrated, by incorporating fluorescein into Betnovate ointment, that the animal does not transfer ointment from the ear to other parts of the body. This separate group of five animals treated with the standard dose of 0·01 ml daily on 10 consecutive days produced fluorescence over the site of application alone.

A ratchet-controlled micrometer screw gauge produced repeatable and accurate measurements of mouse-ear thickness. With the instrument on a vertical bench stand both hands remained free for securing and manoeuvering the mice. Both ears were measured daily.

The following corticosteroids were examined:

Dermovate ointment, 0·05% clobetasol propionate; Betnovate ointment, 0·1% betamethasone valerate; Molivate ointment, 0·05% clobetasone butyrate; Locoid ointment, 0·1% hydrocortisone butyrate; Dioderm, 0·1% hydrocortisone B.P. in a specially formulated water miscible base.

Ointment base, white soft paraffin (WSP) with 2% liquid paraffin added.

The control groups of mice anaesthetized daily received no local application. Measurement of ear thickness was taken on both ears.

Human study. A small pilot study undertaken in the Skin Department on adult normal volunteers established an accurate, reliable method for measuring skin thickness change following topical corticosteroid use.

The normal subjects, whose results form the basis of this report, came from the Central Post Office, adjacent to the hospital.

Skin-fold thickness measurements were taken at points on the flexor surface of the forearm, well away from the wrist and antecubital fossa to minimize disturbance of dressings by work or movement. The readings were taken at 7-day intervals on three occasions.

At each application site a small tattoo was made with colloidal carbon ink. Treatment sites were aligned along the median longitudinal axis of each arm. Marks were made through holes in a perforated metal strip. Each hole in the strip was spaced regularly 3 inches from the next. The end of the metal strip, when placed at the wrist joint crease with the joint flexed, enabled the carbon mark to be found easily.

In the first experiment (Group 1) two marks were made on each arm and skin-fold thickness measurements taken. Constant and identical readings could be made in many of the subjects using the ratchet-controlled micrometer as in the animal experiments. If this was not possible, then a mean value was obtained from several readings.

In three people it proved impossible to raise a fold of skin from underlying tissues, and these subjects were omitted from the analysis. As the result of unassociated illness or of plaster reaction some volunteers were removed from the study. This left eighteen people in the first group and nineteen in the second.

After measurement of skin-fold thickness, 0·05 ml of ointment was expressed onto an Al Strip dressing from which the gauze had been removed. Micropore tape secured the foil to the flexor surface of each arm. Dressings were renewed weekly after measurement at each site. In order to assess the spread of corticosteroid ointment with this method fluorescein dye was incorporated into Betnovate ointment. One week later the occluded site, when examined under Wood's light, showed a clearly demarcated square. Fluorescence was confined to the outline, and area covered by the Al Strip. Ointments were labelled from 1 to 4 in the first group and the application made over marks numbered similarly 1–4. No attempt was made to alter the application site for each numbered steroid within Group 1. The sites were altered in the second human group.

Group 1. In this group the final number was eighteen people, 2 female and 16 male. Ages ranged from 19 to 61 years (mean 44·6 years).

The following corticosteroids were applied:

Molivate ointment, 0·05% clobetasone butyrate; Haelan ointment, 0·0125% flurandrenolone; Ultradil ointment, 0·1% fluocortolone pivalate and 0·1% fluocortolone hexanoate.

Unmedicated base, white soft paraffin (W.S.P.) with 2% liquid paraffin added.

Standard preparations were compared. Treatment 1 was applied at site 1 and so on.

Results were expressed as the mean percentage skin thickness change for each application.

Group 2 comprised nineteen people, 4 females and 15 males. Ages ranged from 26 to 56 years (mean 45·2 years). Ointments were used in this experiment. Dioderm contains a specially formulated water-miscible base. Six preparations were assessed for skin-thinning potential, Molivate, Betnovate, Dermovate, Locoid, Dioderm and unmedicated ointment base.

These compounds were the standard ones as supplied by the manufacturer and obtained from the hospital pharmacy and were identical to those in the animal study. The site of application was varied within this group. Changing site did not produce statistically significant differences between right or left arm, proximal or distal placement on the same side. Readings and the fresh application of steroid were made at weekly intervals and on three occasions.

RESULTS

Animal study: mouse ear. It was noticed in the initial experiments that, following application of corticosteroid to one ear, the other became thinner. This effect has been consistent and is presumably due to systemic action following absorption. Applying ointments of low potency to the left ear produces appreciable change on the right, (Fig. 1):

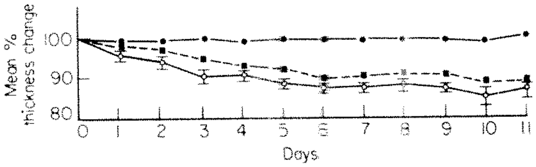

FIGURE 1. Change in thickness of treated and untreated control mouse ears induced by hydrocortisone ointment. ○——○ Left treated ear HC; ■ - - - ■ right untreated ear; ●——● controls anaesthetic alone.

The ointment base has produced moderate thinning of the treated ear (Fig. 2). Thus it proved impossible to assess skin-thinning activity by applying the active compound on one ear, and the base to the other. Instead each compound has been compared with the untreated ears in a control group of mice given ether anaesthetic alone.

The anaesthetic, given daily for up to 3 weeks or more, does not produce ear thinning (Fig. 3). The difference in thickness between the two ears of an untreated mouse is not significant.

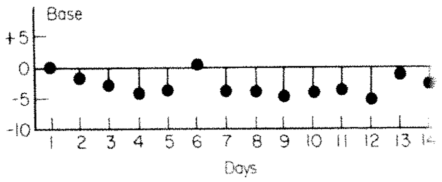

FIG. 2

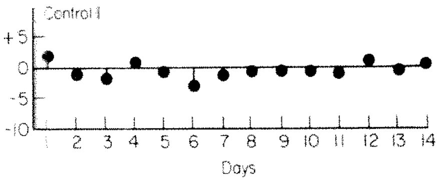

FIG. 3

FIGURE 2. Thinning of mouse ears (percentage change from control) induced by unmedicated ointment base.

FIGURE 3. Lack of effect on thickness of mouse ears of daily anaesthetizing with ether.

In the mice most corticosteroids have produced their maximal thinning effect within 5 days (Table 1). Beyond this time varying patterns of change are seen. Betnovate ointment- and Dermovate ointment-treated ears have in all experiments shown a recovery from their initial thinning. This effect is of some interest and will be the subject of a further communication. We believe that in the light of our present knowledge a second point of comparison would cause confusion.

We have chosen, therefore, to compare the maximal mean values of ear thinning obtained within the first 5 days for each of the treatments. The model when used in this way produces a rank order. Some corticosteroids when applied daily have a marked thinning potential whilst others are clearly demarcated by producing only a moderate effect. Between these two extremes is a group whose activity tends to overlap that showing the greatest change.

Human study

Group 1. After 1 week all ointments applied under occlusion had produced thinning of forearm skin. Two-week measurements showed a further decrease in skin thickness on corticosteroid-treated sites. The ointment base-treated skin had increased in thickness when compared to the reading after

TABLE 1. Mean percentage thinning of mouse ears after 5 days, treatment with topical steroid and control ointments

Ointment	Maximum (mean) thinning within 5 days (%)
Dermovate	−14·7
Locoid	−13·0
Molivate	−11·6
Betnovate	−11·2
Dioderm	−8·2
Base	−4·1

1 week. A clear difference is seen between the moderate activity of base and Ultradil and the more obvious skin thinning produced by Haelan and Molivate (Fig. 4).

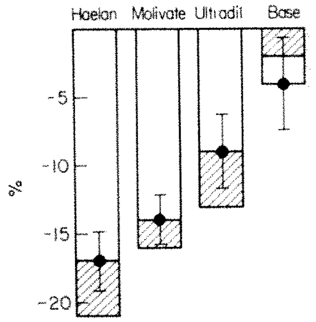

FIGURE 4. Percentage change in skin thickness in humans (Group 1:18 people) after two weekly applications, under occlusion, of three topical steroids and control. Vertical bars, s.e. mean; open column, 1st week; hatched column, 2nd week.

Group 2. All compounds examined in this group produced skin thinning within a week (Fig. 5). Measurement after 2 weeks' application showed a varied pattern of response. Dermovate- and Betnovate-treated sites had increased in thickness beyond the 1st week readings. Dioderm-treated skin showed a similar change. A rank order of thinning is established, again groups can be separated.

Friedman's two-way analysis of variance applied to each of the treatment groups showed a clear difference between treatments, significant at the 0·1% level. Tests of comparison between individual compounds were not made. Fig. 6 demonstrates a parallel response between human skin and mouse ear thinning after continued topical corticosteroid application.

DISCUSSION

In both the human and the animal studies, changes in skin thickness have been used as a measure of atrophy following application of corticosteroids. Patients with Cushing's syndrome have a measurably thinner skin than controls (Black, Bottoms & Shuster, 1970; Arho, 1972).

ACTH administered to rats has thinned their skin (Baker *et al.*, 1948). Adrenocortical extracts

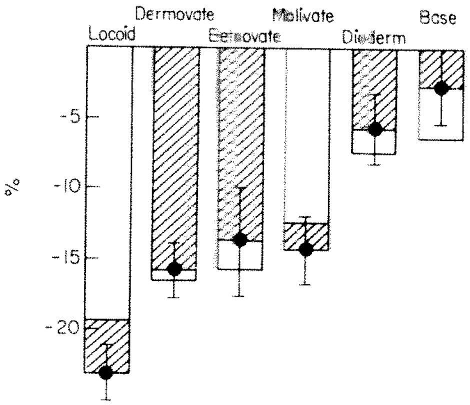

FIGURE 5. Percentage change in skin thickness in humans (Group 2:19 people) after two weekly applications, under occlusion, of five topical steroids and control. Vertical bars, s.e. mean; open column, 1st week; hatched column, 2nd week.

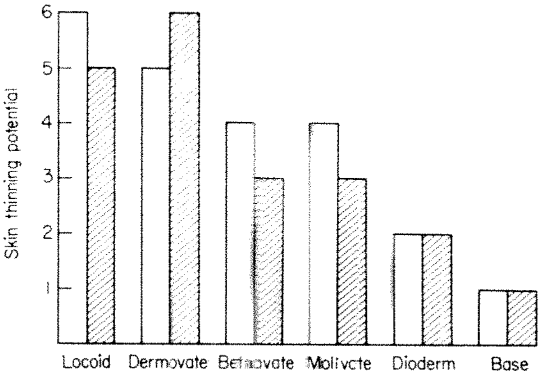

FIGURE 6. Percentage decrease in skin thickness in the mouse ear (after 5 days) compared with the human forearm (after 7 days). Open column, man; hatched column, mouse.

applied to rat skin produce thinning (Baker & Whitaker, 1948; Castor & Baker, 1950). We cannot assume, however, that atrophic-looking skin is measurably thinner than the normal skin of controls (McConkey, Fraser & Bligh, 1965). The appearance of skin atrophy is associated with changes in dermal connective tissue (Castor & Baker, 1950; McConkey *et al.*, 1967; Shuster, Raffle & Bottoms, 1967).

The micrometer method measures whole skin thickness and therefore includes dermal changes. Some workers have used epidermal thinning to assess the atrophic potential of topical corticosteroids (Winter & Wilson, 1976). Other methods of skin measurement are few, and not applicable to normal volunteers on a wide scale. Histological measurement of dermis is difficult (Castor & Baker, 1950). In the preparation and examination of biopsy material wide sources of error may occur. Radiographic measurement of skin thickness in normal people poses a difficult ethical problem, when each individual may be exposed to radiation on several occasions.

Our results have shown that with a ratchet control micrometer, measurement is reproducible and consistent on both mouse ear and human skin. The mouse ear is particularly well suited to measurement with this instrument. Individual mice in control groups would often have identical ear measurements on successive days.

Standard errors of the mean levels (Fig. 1) are of the small order seen throughout the animal experiments. A measurable change in thickness occurred in all ears treated with topical corticosteroids. The slope of each graph showing percentage thinning against time was reproducible in any group of mice when the same steroid was used.

The cause of the recovery from initial thinning produced by Betnovate ointment and Dermovate ointment is uncertain. It may well represent changes in dermal connective tissue similar to those described in human skin (McConkey *et al.*, 1965). Further histological investigation will help to solve this problem. Du Vivier & Stoughton (1975) have shown tachyphylaxis to the vasoconstrictor effect of corticosteroids applied to human skin. We cannot be sure that we are witnessing tachyphylaxis in the animal model or in human skin whilst measuring atrophy.

We have noted moderate thinning of the mouse ear after the continued application of WSP. Epidermal thickening has been reported after the application of this base to guinea-pig skin (Sarkany & Gaylarde, 1973). Christophers (1970) noted thickening together with a decrease in epidermal activity. We do not know what action the base has within the dermis. When applying the base to human skin, rethickening was evident after 1 week.

In the human, recovery from the initial skin thinning was seen at 2 weeks with Betnovate ointment, Dermovate ointment and Dioderm. It is possible that the first two may have a more profound effect on dermal connective tissue, and that increasing fluid content could account for part of this change.

All treatments have been applied to each human volunteer; each group of mice received only one application. Despite these limitations, which we cannot obviate in the animal model, the findings have been compared with the human results.

CONCLUSIONS

Skin thinning following the topical application of corticosteroids occurs quickly, and the micrometer method enables us to detect this change.

The method is reliable, non-invasive and safe. Changes in both epidermis and dermis occur with clinical atrophy. How much each component of the skin contributes to what we see as atrophy in the patient remains for debate. The method is able to detect changes in both layers of the skin.

Most clinicians would regard atrophy as synonymous with skin thinning occurring after steroid treatment. It is demonstrated that, with their continued topical use, some of these compounds may cause the skin to rethicken.

ACKNOWLEDGMENTS

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DISCUSSION

PROF. F.J.G.EBLING (SHEFFIELD): The interpretation you put on these results when you say that continued application causes the skin to rethicken seems to be unjustified. What you are really saying is that continued application does not result in skin remaining thin. If you are really going to claim there is a rethickening, you would need as a proper control to have skin in which treatment had been stopped, and then you would have to compare these factors if you are really claiming a rethickening has occurred.

DR. J.D.KIRBY: I do not know how important it is. I think it may well represent changes in fluid content, for example.

DR A.JARRETT (LONDON): You get thinning with the control, and in our experiments and those of others it has been found that a whole range of bases caused thickening.

DR J.D.KIRBY: We are talking about the effects on the epidermis; what we do not know is what effects these compounds have on the dermis. With our method we are looking at both layers of the skin. Until we know more about the action of these very complex compounds on the dermis, the answer to this sort of problem has to remain in abeyance.

DR J.L.BURTON (BRISTOL): One possible criticism of the mouse as a model for this type of study is that the epidermis is so thin compared with that of the human epidermis that you may be giving it maximum stimulation with any steroid preparation, and I wonder whether this accounts for the relative lack of discrimination which your first experiments showed.

DR J.D.KIRBY: I think this is right. When you look at the histology in some cases the epidermis has almost disappeared.

DR C.N.D.CRUICKSHANK (BIRMINGHAM): We have to be very careful about our thinking because you are probably measuring two effects: one on the epidermis and one on the dermis. Of the two, it is very likely that the dermal effect is the larger one because of the relative proportions of dermis and epidermis. It has been suggested that the effect of epidermal atrophy has to do with interference with cell replication. This might well be an over-simplification of that problem, but in any event, it is possible that these drugs have two quite distinct and separate actions on the epidermis and on the dermis.

DR R.A.MAIN (ABERDEEN): You showed clearly that hydrocortisone butyrate has a marked thinning effect. My understanding is that hydrocortisone butyrate is reputedly broken down into hydrocortisone and butyric acid. It seems unusual therefore that one sees such an atrophic effect.

DR D.D.MUNRO (LONDON): Although it has been claimed that hydrocortisone butyrate is broken down to hydrocortisone and butyric acid, clearly this is not so. Hydrocortisone butyrate is an extremely potent steroid and goes through the skin and into the circulation as hydrocortisone butyrate. That is why you get adrenal suppression. We have done some specific studies using the pure preparation and incubating it with human sera, and it becomes clear that it is not metabolized as the manufacturers indicated it was.

DR J.R.S.RENDALL (OXFORD): Could it not just be that traumatizing the skin by rubbing in both the series you did was the cause of thickening?

DR J.D.KIRBY: It has not been a constant feature with all preparations tested so I do not think the friction produced by rubbing has caused this rethickening.

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Atrophy of the skin

THE CLINICAL PROBLEMS

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The topical application of corticosteroids has enabled the control of so many dermatoses that one should preserve a balanced view on the occasional harmful effects which occur as a result of their abuse.

There is a wide awareness amongst dermatologists and family practitioners that temporary damage may occur from the injudicious use of topical steroids but the damage may not *always* be reversible. New examples of atrophy produced by steroids, first noticed by Epstein, Epstein & Epstein (1963) as striae in the groin and axillae, still appear. It is common for one member of a family or a friend to pass on an ointment which has done them good and if new prescriptions are obtained through the receptionist steroids may be used for many months without a doctor seeing the patient. Within a few months the continued use of a powerful steroid may have produced atrophy. Fortunately this is usually, but not always, reversible.

The atrophic changes which occur from the injudicious use of steroids include atrophic striae. These certainly do not occur in every patient when powerful steroids are used, but they are more liable to occur in childhood and particularly at puberty. In many adolescents there is already a tendency to striae formation as a result of the outpouring of growth hormone and the application of topical steroids at this time gives an added boost to what is already occurring.

Very similar to striae is the superficial atrophy induced by strong steroids applied to skin surfaces in contact with each other, where sweating produces increased absorption, simulating the effect of polythene occlusion. This may occur under the breasts.

A similar change can be seen in the perianal region when powerful steroids are used for long periods for the control of pruritis ani. Telangiectasia, indicating loss of dermal collagen, occurs and the patient who originally complained of pruritis now complains of soreness and burning. This is the result of erosion of the thinned epidermis and is aggravated by exercise. The friction of walking rubs off the atrophic epidermis and patients complain bitterly of pain rather than itching.

One can therefore formulate cautionary rules about the use of strong steroids in these cases. The treatment should be continued for only a short time but in many of them the stronger form of steroid is probably not required. It must be remembered that if strong steroids are put into the patients' hands they are likely to continue to use them, despite medical advice, because rebound recurrence of inflammation may occur when the treatment is stopped. One should be especially careful in prescribing strong steroids for children and adolescents.

The second area which appears particularly vulnerable to atrophy from steroids is the face. Presumably, and I have no proof of this, it is because steroids penetrate the skin of the face more readily. The ease with which the face can be treated may also be a factor and it is noticeable that most patients with steroid-induced atrophy of the facial skin are female.

A variety of syndromes occur. First, telangiectasia in patients who show no predisposition to rosacea. As is so often the case there is abuse of the steroid application before this occurs. In men, telangiectasia can follow the use of steroids on folliculitis. The skin gradually becomes redder but there may be no other change.

Patients with atopic eczema seldom develop atrophy of the face, possibly because the epidermis is thickened, and penetration of the steroid to the dermis is insufficient to cause destruction of collagen. However, occasionally it does occur, but in young people any telangiectasia produced appears to be reversible. Recovery depends on age rather than duration of treatment, and it may well be that in the older age groups topical steroids are only accelerating the natural process of skin collagen damage by light.

I recently followed up the course of a woman aged 36 years who had controlled atopic eczema of her face by using betamethasone valerate cream under her own make-up for 10 years. She developed severe telangiectasia over the whole of the treated area. She was first seen in July 1973. Cessation of the use of the steroid did not produce a significant improvement and her facial skin remains telangiectatic and atrophic-looking in 1975.

It has been widely accepted, though not universally, that rosacea with its tendency to telangiectasia should not be treated with powerful steroids. Fourteen cases were originally described (Sneddon, 1969) in whom telangiectasia had extended to the hair margin and it would appear that this was due to atrophy occurring in the collagen, allowing the blood vessels to become more visible. In the majority of patients this condition is reversible. I personally believe that strong steroids should not be used on the face for the treatment of rosacea or even mild dermatitis. Perioral dermatitis which may also be connected to the use of strong steroids cannot be described as an atrophy and is not within the brief of this paper.

I would like to put in a plea that steroids of all kinds should be avoided in the treatment of healing ulcers. Many mixtures of corticosteroids and antibiotics are advocated for the treatment of gravitational and other ulcers. Whilst they may control infection, and also the surrounding dermatitis, steroids are lethal to exposed fibroblasts and cause blanching of blood vessels; dry indolent ulcers which show no sign of repair after months of treatment with steroid creams are still seen in our clinics. This is one form of atrophy which is undoubtedly avoidable.

Finally there is one point on which I have seen no comment, and that is the penetration of steroids through atrophic skin: one would expect it to be increased. As the epidermis and dermis become thinned a larger and larger amount of steroid must penetrate to the dermal blood vessels and thus may have systemic effects. We know that, given reasonable care, adrenal suppression does not occur to any great extent with the topical use of steroids. I have, however, had one patient whose mother was obsessional and treated her 11-year-old child, who had mild psoriasis, with 120 g of betamethasone valerate ointment under polythene occlusion weekly. The girl developed striae so severe that the subcutaneous fat ruptured through them. When she was first seen it was not appreciated that she was dependent on the steroid penetrating the skin to replace her suppressed serum cortisol and when the betamethasone valerate cream was discontinued she died from adrenal failure.

We must therefore be aware that probably more steroid is absorbed if skin atrophy has developed and be particularly on our guard if there is a possibility of adrenal suppression, as cover with systemic steroids may be necessary when withdrawing topical treatment.

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DISCUSSION

DR H.BAKER (LONDON): In the discussion we have heard no mention of Propaderm which I believe is chlorinated rather than fluorinated. Could you comment on the clinical atrophogenic potential of this?

DR SNEDDON: A fair number of patients in our area have used Propaderm and have produced the same sort of appearance on the face that one gets with Betnovate and Synalar. I do not think it has anything to do with fluorination. It is the potency of the steroid.

DR J.A.COTTERILL (LEEDS): Steroid ointments are passed over the garden fence, from one lady's handbag to another, and from one typewriter to another. I do not think that manufacturers can absolve themselves from recognizing this fact.

DR E.S.SNELL (LONDON): There is wording on the tube saying 'Use only in accordance with a doctor's instructions'. There might be an arbiter here, on who is responsible for damage to patients, and that is the law court. If a patient obtains a tube of anything over the fence which is normally obtainable only on prescription and damages herself, she has no one to blame but herself. If she damages herself when using it according to a doctor's prescription and directions, the doctor might be liable. The pharmaceutical manufacturer would not be liable unless the product was faulty in some way, or he had not made available to doctors all the evidence that was generally available at the time.

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Topical steroids and bacterial infection

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My own interest in this subject began with the finding that a large number of skin patients had been treated with a diluted steroid cream heavily contaminated with *Pseudomonas aeruginosa* (Noble & Savin, 1966). We tested samples of dilutions of the same steroid cream from eight other hospitals, and three carried profuse growths of bacteria, two with *Pseudomonas* and one with an *Achromobacter*. The problem may then have been a common one, yet the harm done was probably minor as ours was the only outbreak of clinical infection to be reported.

Patients infected in this way developed pustules, vesicles and multiple small ulcers (Savin, 1967a). The obvious lesson was in pharmacy hygiene (Blacow, 1972); but there were lessons also for those interested in the mechanisms of skin infection. The organisms had been applied to skin already damaged by disease, making infection easy. *Pseudomonas* does not survive well on the skin and even in the cream we could not recover it from healthy, normal skin 1 h after application, although much longer survival times have recently been reported (McBride, Duncan & Knox, 1975). The high humidity of occlusive dressings, later confirmed as predisposing to *Pseudomonas* infections (Hojyo-Tomoka, Marples & Kligman, 1973) and the suppression of the competing Gram-positive flora by clioquinol (Vioform) incorporated in the cream, helped the organisms survive and added to the severity of the infections.

This outbreak was only one of a series of worrying episodes in which patients have been infected by contaminated medical materials including the sphygmomanometer and barium enemata. The clinical aspects of the microbiology of topical preparations was reviewed in depth (Savin, 1967b) and has continued to give rise to concern particularly in the field of cosmetics and eye preparations (Wargo, 1973).

The role of the steroid ingredient in the cream was hard to evaluate in our series, but, unlike most organisms, whose metabolism is enhanced by low concentrations but inhibited by high concentrations, *Pseudomonas* can use glucocorticoid esters as a metabolic substrate. This explains its increased metabolic activity even in the presence of high steroid concentrations (Raab, 1971). However, there is no clinical doubt that topical steroids do only harm if applied alone to frank skin infections such as impetigo or ringworm. Faulty diagnosis allows this to happen unpleasantly often, as is shown by the many cases referred by general practitioners to skin clinics. The literature on this topic is thin, when contrasted with the number of patients seen, as to set up a prospective series would be unethical, and no one likes to admit to mistakes in public. 'Tinea incognito' (Ive & Marks, 1968) was one exception to this.

It is no criticism of the steroids themselves if they give rise to complications when used wrongly. There are many theoretical reasons why they might worsen an infection (Table 1) and this raises the question of whether topical steroids should be used only when combined with anti-bacterial agents in

TABLE 1. Possible reasons for topical steroids worsening infections

1. Suppression of inflammatory response to infections
2. Vasoconstriction with slower mobilization of defences
3. Increased hydration of stratum corneum by base or occlusion
4. Decreased epidermal turnover with reduced shedding of scales and surface organisms
5. Stimulation of metabolism of pathogens (especially *Pseudomonas*)
6. May depress activity of antimicrobials (*in vitro*)
7. May obscure diagnosis

the treatment of skin diseases. Many doctors must take this sweeping line as, in 1966, 48% of topical steroid preparations sold in the United States contained additional anti-infection agents (Lloyd, 1969). However, blanket notions are used to cover up loose thinking and it would be foolish not to be, aware of the many problems associated with topical antibacterials (Table 2).

TABLE 2. Some disadvantages of topical antibacterial agents

1. Emergence of resistant pathogens
2. Suppression of normal flora whose antibiotic production inhibits pathogens
3. Contact sensitization, followed by eruptions if used systemically
4. Absorption may (a) lead to dangerously high blood levels (hexachlorophane)
   (b) affect thyroid function tests (clioquinol)
5. May decrease penetration of topical steroids
6. Skin staining

One rational approach is first to group skin diseases by the role that micro-organisms play in them (Table 3) and then to consider each group in turn. It is self-evident that group 1 diseases need antibacterial agents; but are steroid antibiotic mixtures as effective as the antibiotic alone? Marples Rebora & Kligman (1973) studied experimental staphylococcal infections in man. They found that creams containing only antibiotics (gramicidin and neomycin) eliminated the organism in most cases,

TABLE 3. A classification of skin diseases by the role played by bacterial pathogens

Group		Need for steroid–antibiotic combinations
I	Organisms are responsible for the disease, e.g. impetigo, furunculosis	Nil. Antibacterials alone
II	Skin disease with added bacterial infection, e.g. impetiginized eczema	Dictated by physician's preference largely
III	Skin disease with bacterial colonization not apparent clinically, e.g. psoriasis, atopic eczema	Only if a danger of epidemics from infected scales, or if occlusion used
IV	Skin diseases not colonized by bacterial pathogens, e.g. granuloma annulare	Nil

and in the other patients reduced the pathogens to very low levels. When the antibiotics were incorporated in a cream with triamcinolone acetonide, the organisms were killed just as well, and the clinical signs of infection were suppressed rather better. In this sense the combined cream was the better, though one would not recommend it in clinical practice.

The treatment of group II diseases needs a clinical and not an experimental solution. The obvious line of attack, treating comparable lesions on opposite sides of the same patient with a steroid alone or with a steroid–antibiotic mixture (Davis, Fulghum & Taplin, 1968), is affected by the way in which topical anti-bacterial agents will spread over the skin surface in amounts great enough to suppress bacterial growth in distant areas (Marples & Kligman, 1973). Failure to find a different effect on the two sides of the body must be meaningless because of this, although there may still be a genuine major difference. A better method is to treat each patient with a single preparation. Lloyd (1969) treated patients' clinically infected dermatoses with either triamcinolone acetonide cream or the same cream containing neomycin. No clinical difference—surely the most important criterion—was found between the two groups despite the more efficient elimination of staphylococci in the group treated with the combined cream. Perhaps by cutting down scaling and exudate the topical steroids made the affected areas less suitable for bacterial pathogens.

If these are carefully looked for, many dermatoses in group III will yield bacterial pathogens, sometimes in large amounts; psoriasis (Noble & Savin, 1968) and atopic eczema (Selwyn & Chalmers, 1965) are good examples. Occasionally the massive desquamation of scales loaded with organisms had led to epidemics of infections in others (Payne, 1967). A case exists in group III diseases for using antibiotic–steroid combinations, rather than steroids alone, if there is a danger of this, for example in a surgical ward, or perhaps if polythene occlusion is to be used. Otherwise the use of these combinations may be no more than a device to relieve the, often false, anxiety of the prescriber.

There is, of course, no justification for the use of steroid–anti-bacterial combinations in group IV diseases.

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DISCUSSION

DR E.M.DONALDSON (STOKE-ON-TRENT): I should like to ask about the place of neomycin in proprietary preparations.

DR E.S.SNELL (LONDON): We produce steroids with three different anti-bacterials: neomycin, clioquinol and tetracycline. The one people use most is neomycin. It has been said that neomycin is a potent sensitizer. I think it is a very weak sensitizer, but the point is it is used so widely that inevitably this question of sensitized patients turns up. Possibly the only weaker sensitizer is tetracycline, but that is not such a useful antibacterial agent. The other point in neomycin's favour is that it is not used systemically.

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The use of topical steroids of differing potency in the management of the atopic child

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The prolonged application of high-potency corticosteroid preparations may produce systemic as well as local side-effects. Therefore the importance of using low-potency preparations whenever possible, particularly in the treatment of infantile eczema, is beyond argument. In many children, however, low-potency steroids may not always be adequate to control the disease and it is important that such children are treated, for a short time at least, with preparations of a higher potency.

There are three techniques that may be used to reduce the total amount of high-potency steroids applied during the treatment of infantile eczema: 1. Maintenance on low-potency topical steroid, switching to a high-potency steroid as the sole application for short periods. 2. The use of a high-potency steroid throughout treatment but limiting its application to the most troublesome areas. 3. The use of a high-potency steroid regularly once a day but using a low-potency steroid for the second application.

The choice of technique depends almost entirely upon the intelligence and co-operation of the parents and/or child. We believe stress should always be laid on the importance of reverting to the use of low-potency steroids as soon as possible.

Such variations in treatment make the assessment of the long-term use of topical steroids extremely difficult, and there are few objective data to support clinical impressions. A new type of trial, which has several advantages over previously reported studies, and which takes into consideration the occasional alteration in the potency of locally applied steroids during treatment, is described. One of the problems of short-term trials is the extreme variability of skin signs and symptoms from day to day, but the perfect study, with daily assessments, would obviously over-burden the clinician. Similar problems occur in the assessment of the management of bronchial asthma and Connolly & Godfrey (1970) successfully introduced a technique of self-assessment using a daily record card. We are endeavouring to do the same in the evaluation of infantile eczema and its response to topical steroids.

A record card covering a period of 28 days has been designed and is filled out by the patient or parent every day. Detailed instructions on how to complete the record card are given so that no confusion should occur, and the card has been so designed that the data obtained can be analysed using a computer. On this card the skin condition is assessed daily, using a very simple scoring system where 0 = clear, 1 = mild and 2 = severe. All treatment applied is recorded and quantified and this applies not only to the corticosteroid under investigation but to any other treatment used topically or systemically.

At the initial interview a clinical history is taken and the relevant data recorded by the clinician on a patient record form. This includes the clinician's assessment of the eczema and information which enables the body surface area involved to be calculated. The trial is a double-blind cross-over study extending over a period of 6 months. Flurandrenolone (0·0125%) ointment (Haelan) is being compared with a new topical steroid clobetasone butyrate (0·05%) ointment (Molivate). The latter has been claimed to be a clinically effective topical anti-inflammatory steroid with a wide margin of safety (Munro & Wilson, 1975). One of these two steroids is randomly allocated to each child for the first 3 months and then the alternative steroid is applied during the second 3 months. Four months after the beginning of the trial the patient's and the physician's preference for treatment are recorded. Adrenal function is being monitored regularly by determining plasma cortisol levels monthly. All the children studied were known to two of the investigators for at least 3 months before entering the trial and were stabilized on flurandrenolone. This trial has now been under way for a little over 6 months and neither parents nor clinicians concerned have encountered any major problems. The advantages of this type of trial are the ability: 1. To assess the day-to-day state of a patient's skin. 2. To determine the quantity of corticosteroid applied per unit surface area per unit time. 3. To assess the results of treatment with one or more corticosteroid preparations over a relatively prolonged period. 4. To note any alteration in the total requirements for short-term high-potency corticosteroids per unit time during the regular use of low-potency preparations. 5. To note any evidence of adrenal suppression by determining monthly plasma cortisol.

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DISCUSSION

DR E.R.M.ROWELL (LEEDS): I should be interested to know what sort of amounts of steroids you have used in these trials.

DR C.F.H.VICKERS: On average a 5-year-old child in this trial is using something in the region of 30–50 g of steroid ointment per week. Many of the children are using as little as 10 g a week. One of them who has minimal eczema and is 8 years old is using 300 g per week. We are not attempting to control the amount the mother is using for obvious reasons.

MISS LYN WILSON (LONDON): In trials where the patients are being carefully supervised, it is difficult to know whether the amounts used are those that would normally be applied. However, we found (Wilson, Williams & Marsh, 1973) that more than 80% of 295 unselected out-patients (that is they were not taking part in any trial) being treated with topical steroids were in fact using less than 30 g a week. So that people using vast amounts probably represent a relatively small proportion of those using steroids.

DR T.C.HINDSON (BRITISH ARMY): One thing we should like to emphasize is what we think is the importance of humidity in the environment in this connection. In 1968 we did a study in Germany and in Singapore, where we had an army of about 500,000 soldiers. We found that 18% of our patients reporting in Germany were children with atopic eczema, and in a similar age and sex population in Singapore it was less than 1%. Although we have no evidence, we attributed this to the effect of humidity. Living in Germany it was noted that in January and February personnel lived in completely dry, centrally heated houses, whereas in Singapore, the relative humidity was 90%. Certainly I have found that increasing humidity cuts down the time of treatment.

DR C.F.H.VICKERS: I am noticing in this trial that there is tremendous seasonal variation.

Reference

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British Journal of Dermatology (1976) **94**, Supplement **12**, 131.

Indications for systemic steroids in dermatology

ALAN LYELL

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Systemic corticosteroid treatment occupies a comparatively small place in the armamentarium of the critical dermatologist, since topical application is preferred whenever possible because of the much reduced risk of serious side-effects. Nevertheless, within a narrow range of indications, systemic treatment has revolutionized management. But, since its effect is suppressive, treatment will have to be continued over a long term, unless the disease being treated is self-limiting. Anxiety over serious, even lethal, side-effects has increased the responsibilities of the therapist, who has to navigate the difficult waters between the Scylla of under-treatment and the Charybdis of toxicity.

The chief indications for systemic corticosteroid treatment are the major bullous diseases and systemic lupus erythematosus. Savin's (1975) study—which incidentally revealed some interesting sidelights on death certification—showed a fall in deaths attributable to the bullous diseases dating from the advent of corticosteroid treatment; and although the sceptical may prefer to believe that these patients died, without blisters, from other causes, possibly iatrogenic, there is no doubt in my mind that the outlook in the major bullous diseases has been transformed and that life expectation in systemic lupus erythematosus has increased. I remember pemphigus vulgaris with horror as a disease that killed the young middle aged, slowly but inexorably, with a distressing amount of pain and discomfort, and with the patient fully conscious of what was happening. Systemic corticosteroid treatment has ended this miserable situation so that the patient can look forward, not only to being freed from the manifestations of his disease, but also to life, albeit within the restrictions imposed by continuing treatment, and always under the shadow of possible serious side-effects.

Side-effects are related to the dose employed and the duration of the treatment. The high doses used initially to gain control—for example in active pemphigus vulgaris 60–120 mg of prednisolone per day by us, and 180–360 mg per day by Lever (1971)—are usually well tolerated, serious side-effects tending to appear later on, after the dosage has been reduced gradually to maintenance levels. If the maintenance dose of prednisolone can be held below 15 mg per day, the chance of serious side-effects developing is reduced. To this end anti-mitotic drugs such as azathioprine and methotrexate are often added, for their effect in reducing corticosteroid requirements. It seems to me possible, however, that confidence in the comparative harmlessness of antimitotic drugs for maintenance treatment, as compared with corticosteroids, may prove eventually to have been somewhat misplaced. Proper supervision of patients implies regular review of the clinical state, weight, urine, blood pressure and electrolytes (and blood counts and liver function tests for those on combined treatment), by the same person, or at any rate by the same team. One way of organizing this in a busy hospital out-patient department is a steroid clinic. An up-to-date steroid card carried by the patient, who is trained to show it to any doctor or dentist whom he may consult, is his chief protection against the hazard of adrenal failure induced by emergency situations. Alternate day treatment is desirable wherever possible, especially in children, as it may help in overcoming the problem of inhibiting growth. Our experience in the steroid clinic is that careful supervision and strict control of the dose to the minimum level required for suppression of disease are very helpful in minimizing serious side-effects, though infections, particularly silent and opportunist infections, pose a serious threat that requires constant vigilance. Patients with a definite history of peptic ulceration or evidence of marked hypertension are accepted for treatment only under pressing circumstances.

The bullous diseases with the exception of benign mucous membrane pemphigoid usually respond well; in fact one reason for an unexpectedly poor response is that this disease has been overlooked; another is if the blisters depend upon occult malignancy.

In lupus erythematosus there may be a problem in knowing when to start treatment, because of symptoms without confirmatory laboratory tests, or *vice versa*. In this situation the symptoms offer a better indication than the tests. The criteria of control of the disease vary from patient to patient, being, for example, the level of the white count in one and the feeling of tiredness in another.

Exfoliative erythroderma is another indication for systemic corticosteroids, but cases secondary to psoriasis are better not treated in this way on a long-term basis. In fact, in our opinion, systemic corticosteroids are justifiable in psoriasis only as a last resort and life-saving measure, for example in uncontrollable pustular psoriasis of the von Zumbusch type.

Systemic corticosteroids can be used in cutaneous reticuloses in conjunction with irradiation and antimitotic drugs. Other collagen diseases (except systemic sclerosis), epidermolysis bullosa, allergic vasculitis, actinic reticuloid, severely disabling atopic eczema and continuing major aphthous ulceration are further indications. Good hair growth may be possible in severe or total alopecia, but continuation of hair growth may depend upon maintenance therapy, and I always remember Twiston Davies' acid comment—better a bald coot than a dead duck.

Depot injections have a place, particularly in intractable senile pruritus, and in severe dermatoses of elderly and terminal patients. They may also have to be used to replace oral treatment in patients whose ability to take the stated dose of tablets is open to question.

The uses mentioned so far have been long-term ones, but systemic corticosteroids also have a short-term use, for example in tiding the patient over an exceptionally severe attack of contact dermatitis or of urticaria (adrenalin is needed for a really rapid effect in urticaria), or for drug eruptions, severe erythema multiforme or toxic epidermal necrolysis. Some severe cases of lichen planus merit a short course, which not only suppresses the symptoms, but often seems to hasten final resolution. Short-term systemic corticosteroids are used much more freely in general practice than in hospital, and it is not unusual to be presented with a patient who is undiagnosable because his eruption has been modified by this treatment. The dermatologist is liable to vent his irritation in various ways, of which this is an example. 'Dear Doctor, your patient has scabies. An acarus was identified under the microscope. I was surprised that it did not appear to be moon-faced. Yours sincerely.'

In summary then systemic corticosteroids have a small but definite place in dermatology for either short- or long-term treatment. Their use, particularly for long-term treatment, puts a special responsibility on the therapist to supervise the patient closely and regularly. While this can be expected to minimize serious side-effects, it cannot eliminate them completely, and death is a possible outcome of the treatment. The indications for treating life-threatening diseases are therefore clearer than those for non-fatal diseases. But each patient's case must be judged on its own merits, in the light of the individual therapist's opinion. Opinions may vary from a rigidly enforced policy of safety first at all costs to a casual *laissez faire* attitude, but I think the patient is best served when discretion is wedded to imagination.

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DISCUSSION

Dr C.Vickers (Liverpool): It is my impression that when you have to use systemic steroids in dermatology, in a life-threatening situation, if you start with very high dosage and rapidly bring it down, you seem to get many fewer side-effects than if you start with a small dose and creep up. Do you think this is true, or is it just a clinical impression?

Dr A.Lyell: I think it is true. What surprises me about the Lever therapy is that he recommends his basic dose as 180 mg per day, and continues for 6 weeks whatever, only then bringing it down. We bring ours down almost as soon as the disease seems to be getting under control.

Dr J.L.Burton (Bristol): I was interested to hear that you feel that the symptomatology is a better guide to when you should treat patients with systemic lupus erythematosus (SLE) than the laboratory test, and I wonder whether you include in the tests anti-DNA antibody titres and complement studies? Our experience is that these offer quite a good guide to the level of activity of the disease, even at a time when the patient may be asymptomatic. We have had a number of patients whom, in retrospect, we wished we had treated earlier. We had abnormal laboratory tests, but we waited until they developed symptoms, and the patients have done badly.

Dr Lyell: We have not found the anti-DNA test very helpful in guiding us as to the treatment. It seems to be more an indication of how the kidneys are doing than the SLE. In a situation where a patient has good clinical evidence of SLE but no positive anti-nuclear factor or other laboratory result, I think such a patient should be treated. It is a more difficult problem when you have a positive laboratory test and an apparently normal patient, and I would still feel that that requires very close watching, but not necessarily treatment at that time.

Dr R.M.Rowell (Leeds): We have done DNA tests and complement levels, and I have found them very disappointing in managing cases. I would agree with Dr Lyell that a fit patient is much better as an indication of success of treatment than all the tests I have been able to study in the last 15 years.

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Corticotrophin and tetracosactrin depot—self-administered for the treatment of eczema

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SUMMARY

The prolonged use of self-administered corticotrophin in the form of 'CMC ACTH' (Crookes) or 'Synacthen Depot' (Ciba) has been considered in twenty-seven patients with severe subacute or chronic eczema. Complications were not troublesome and the therapeutic response was good. Many of the patients were able to control their disease fully without the effort and discomfort of additional local treatment and patients' acceptance of the treatment was high.

There is an extensive literature on the complications of systemic corticosteroids. The major side-effects are well known and include hypertension, fluid retention, electrolyte imbalance, predisposition to infection, peptic ulceration and acne vulgaris. In certain groups of the population particular complications become important, for example impairment of growth in children who require long-term corticosteroid therapy for diseases such as asthma, rheumatoid arthritis or bone-marrow hypoplasia.

The impairment of hypothalamic–pituitary–adrenal (HPA) function which occurs during oral, parenteral or topical corticosteroid therapy may be of great importance in both adults and children. Friedman & Strang (1966) clearly demonstrated that growth impairment which had resulted from prolonged corticosteroid therapy in children could be reversed by the use of corticotrophin (ACTH) injections while maintaining the clinical efficiency of the therapy. Similarly, adult patients who had chronic polyarthritis necessitating corticosteroid therapy, could be changed to ACTH injections and were able to maintain the integrity of the HPA function (Carter & James, 1970a), as demonstrated by stimulation tests and also in showing a normal response to stressful surgical operations (Carter & James 1970b). Other workers, such as Bacon *et al.* (1968), had previously shown similar results, yet some impaired HPA responsiveness was demonstrated by Solem & Brinck-Johnson (1961) and Plager & Cushman (1962), so clearly there is quite marked variation in the HPA suppression or responsiveness which occurs in different individuals, the dosage and length of treatment being important iatrogenic factors.

The naturally occurring anterior pituitary ACTH from human and animal sources contains thirty-nine amino acids, the first twenty-four of which are responsible for its corticotrophic activity; the amino acids numbering 25–33 vary with the species and may well be responsible for the adverse reactions which sometimes occur in man following the injection of animal ACTH. Traces of contaminating protein associated with the ACTH may also cause reactions.

The short half-life of ACTH necessitated the production of depot preparations to make it therapeutically of value. Porcine ACTH in gelatin resulted in a delayed release preparation, but because of the thick consistency was difficult for patients to use for self-administration. The development of a slow release carboxymethylcellulose preparation ('CMC ACTH' Crookes) gave a free-flowing liquid which could be injected with ease subcutaneously.

The development of tetracosactrin, a synthetic polypeptide chain containing the first twenty-four amino acids in the sequence of ACTH, suggested that a preparation free of antigenic stimulant action would give increased therapeutic safety. Tetracosactrin depot ('Synacthen Depot' Ciba) contains this synthetic corticotrophin in a zinc phosphate complex to slow the release of the active component and is readily soluble in water or saline, enabling it to be given subcutaneously or intramuscularly, though irritation with an indurated nodule may follow its subcutaneous use.

The risks of antibody formation to tetracosactrin depot compared with porcine ACTH have not proved as low as had been suspected on theoretical grounds, and in a study of circulating antibodies Ratcliffe, Pritchard & El-Shaboury (1969) showed that the proportion of subjects with significant titres in the three groups was: ACTH in gelatin 52%, ACTH in carboxymethylcellulose 13% and tetracosactrin in zinc phosphate suspension 83%.

More recently anaphylactoid reactions have been reported with Synacthen Depot, and the Committee on Safety of Medicines (1975) warned of the risk of reactions, including deaths, which had occurred with this compound and suggested that patients should be observed for 1 h after the injection and that self-injection by patients should not normally be recommended. Clearly therefore the treatment carries some risk, but the alternatives, e.g. systemic corticosteroids or extensive use of potent corticosteroid topically, also carry risks.

INVESTIGATION—PATIENTS AND TREATMENT

Over the course of 7 years, twenty-seven patients varying in age from 9 to 66 years (mean 33·6 years) were prescribed self-administered corticotrophin for the treatment of severe subacute or chronic skin disease. The group consisted of 11 female and 16 male patients, all of whom suffered from eczema. Eight cases were classified as having constitutional eczema and the other nineteen had atopic eczema.

Despite some initial reluctance, all the patients found it easy to give themselves the drug by deep subcutaneous injection, though two patients stopped the therapy later. The first, a 22-year-old woman, disliked the tender subcutaneous lumps, and the second patient, a 61-year-old alcoholic with a difficult temperament, preferred to control his disease with prednisone tablets and took 25 mg on alternate days.

Ten patients used 'CMC ACTH' and seventeen used Synacthen Depot. The initial dose of 'CMC ACTH' was 60 units on alternate days, the dose and interval between injections being increased or decreased according to the therapeutic response. Patients who used Synacthen Depot started with 2 mg and continued with 1 mg on alternate days.

Within approximately 3 weeks all the patients using Synacthen Depot were maintained on 1 mg every 3rd day, or less frequently, whereas only five of the ten patients using CMC ACTH were maintained on 40 μg every 3rd day, the others requiring 60 or 80 μg every 3rd day.

The intervals between injections were increased and the dose reduced, a slight exacerbation of symptoms being treated by a reduction in the interval between injections.

Therapy was discontinued as soon as the skin disease could be controlled easily by small amounts of corticosteroid ointments, but of the twenty-five cases who remained in the series, the number able to discontinue treatment were as follows:

Within 3 months, three patients; within 6 months, two patients; within 12 months, three patients; within 18 months, three patients.

Of the remaining fourteen cases, one was able to stop treatment after 3¼ years, while the rest continued treatment. Though some have so far received therapy for less than 18 months, the dose and interval between injections have remained steady suggesting that treatment will be maintained over a prolonged period.

The complications of treatment were minor. No patient had symptoms suggestive of an allergic reaction; the most common complaint was of tender nodules at the sites of Synacthen Depot injections. Three patients complained of 'moon face' and two developed transient glycosuria which was controlled when the dose was reduced. Approximately half the patients were aware of increased appetite and had to reduce their food intake carefully to avoid weight gain. Other symptoms which occurred in individual patients included 'a sense of well being', 'restlessness at night', 'unbounding energy', and an increased incidence of folliculitis lesions in a 26-year-old man who had a seborrhoeic skin.

DISCUSSION

Despite the warnings of anaphylactoid reactions, none of the group of patients treated has shown any sign of this. The patients have found it easy to administer the injections. No great difference has been demonstrated in the therapeutic efficiency of the CMC ACTH compared with Synacthen Depot, though the interval between injections at comparable states of the patients' skin disease has tended to be longer with the Synacthen Depot. This is consistent with the longer cortisol response to Synacthen Depot, 1 mg giving a raised blood cortisol for about 36–48 h, whereas the cortisol response to 40 μg of CMC ACTH returns to normal in 20 to 24 h.

The disadvantage of injection compared with oral or local therapy is obvious, but prior to the corticotrophin treatment, many patients were using excessively large amounts of corticosteroids topically with resulting skin atrophy, while others had previously required repeated courses of prednisone.

The great advantage in children who suffer from such severe eczema, or the atopic eczema-asthma syndrome for which they would otherwise require systemic corticosteroids, lies in the evidence of a greatly reduced risk of impaired growth when corticotrophin is used.

Large quantities of potent corticosteroids have been demonstrated to cause stunted growth in young children (Munro, 1975) and in the very small proportion of cases where this risk combined with those of skin atrophy exist, ACTH therapy may be indicated.

Clearly the warnings of the Committee on Safety of Medicines regarding anaphylactoid reactions must not be ignored, but if patients are warned to avoid repeated injections if they develop unusual symptoms, and to remain in company with another person for 1 h after the injection, the risk must be minimal.

Many of the patients enjoyed the freedom of being able to avoid the use of messy ointments on their skin, in some cases for the first time in their lifetime.

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RONA M. MACKIE The immune response in human malignant melanoma
C. L. HARMER The radiotherapy of melanoma

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R. J. G. RYCROFT, H. VALDIMARSSON, L. H. BANNISTER AND R. S. WELLS Chronic muco-cutaneous candidiasis of late-onset, thymoma and myopathy. A report of four cases
BARBARA J. LEPPARD AND H. J. R. BUSSEY Gardner's syndrome with epidermoid cysts showing features of pilomatrixomas

Special Article

M. W. GREAVES AND S. YAMAMOTO The immunopharmacology of the immediate allergic response in skin

Clinical Meetings of the St John's Hospital Dermatological Society

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British Journal of Dermatology, Volume 94, Supplement 12, March 1976

CONTENTS

PAGE

Introduction 1

Steroid Structure and Steroid Activity 3
J. ELKS

The Pharmacological Properties of Corticosteroids in Relation to Clinical Efficacy 15
E. S. SNELL

Acute Tolerance to Effects of Topical Glucocorticosteroids 25
ANTHONY DU VIVIER AND RICHARD B. STOUGHTON

The Clinical Assessment of Topical Corticosteroid Activity 33
LYN WILSON

The Treatment of Vitiligo with Topical Corticosteroids: Light and Electronmicroscopic Studies 43
STANLEY S. BLEEHEN

The Place of Intralesional Steroid Therapy in Dermatology 51
JULIAN VERBOV

Steroids and Oral Disease 59
THOMAS LEHNER

The Use and Interpretation of Tests for Steroid Impaired Hypothalamic–Pituitary–Adrenocortical Function 61
J. LANDON, E. SNITCHER AND LESLEY REES

The Effect of Percutaneously Absorbed Steroids on Hypothalamic–Pituitary–Adrenal Function after Intensive Use in In-patients 67
D. D. MUNRO

Measuring Adrenal Function in Out-patients Using Topical Corticosteroids 77
C. G. SPARKES

Corticosteroids and Pustular Psoriasis 83
HARVEY BAKER

The Response of Psoriasis to Betamethasone Valerate and Clobetasol Propionate—a 6-Month Controlled Study 89
M. F. CORBETT

The Effect of Corticosteroids on Cell Turnover in the Psoriatic Patient: a Review 95
PETER GOODWIN

Clobetasol Propionate Ointment Compared with Dithranol in Lassar's Paste in the Treatment of Psoriasis 101
PHILIP J. MARRIOTT AND D. D. MUNRO

Experimentally Induced Steroid Atrophy in the Domestic Pig and Man 107
GEORGE D. WINTER AND J. L. BURTON

Steroid-induced Atrophy in an Animal and Human Model 111
JOHN D. KIRBY AND D. D. MUNRO

Atrophy of the Skin—The Clinical Problems 121
I. B. SNEDDON

Topical Steroids and Bacterial Infection 125
J. A. SAVIN

The Use of Topical Steroids of Differing Potency in the Management of the Atopic Child 129
C. F. H. VICKERS, S. C. CHARTERS AND S. R. WALKER

Indications for Systemic Steroids in Dermatology 131
ALAN LYELL

Corticotrophin and Tetracosactrin Depot—Self-administered for the Treatment of Eczema 135
D. D. MUNRO

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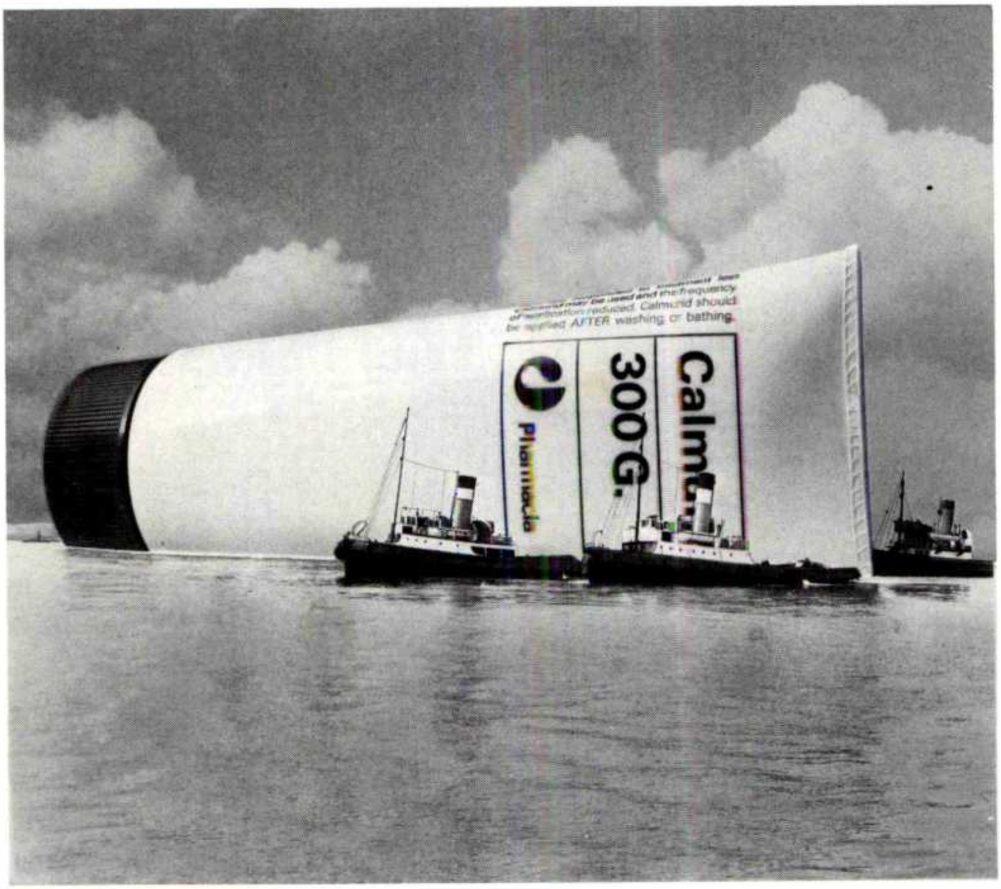

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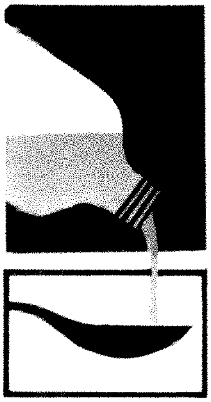

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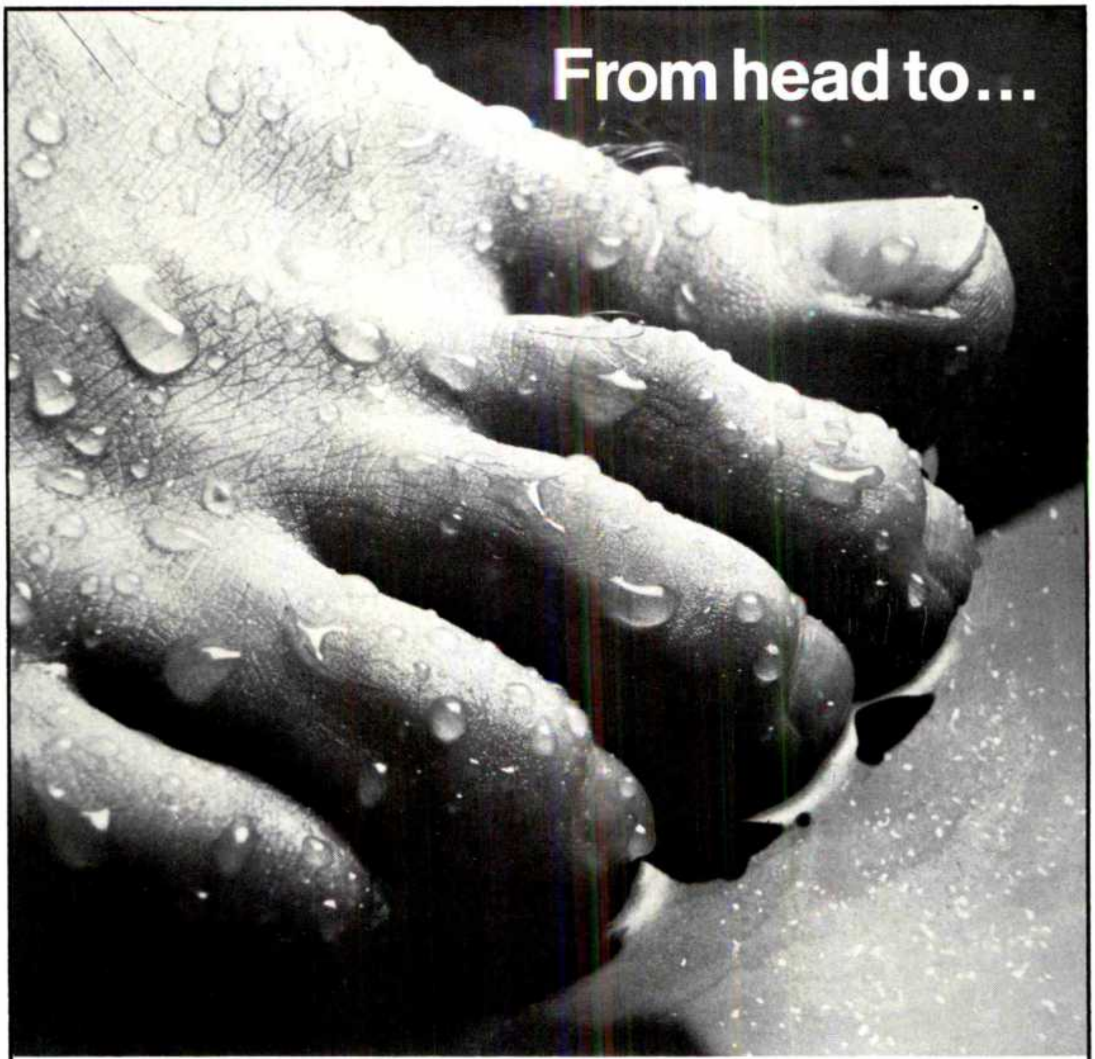
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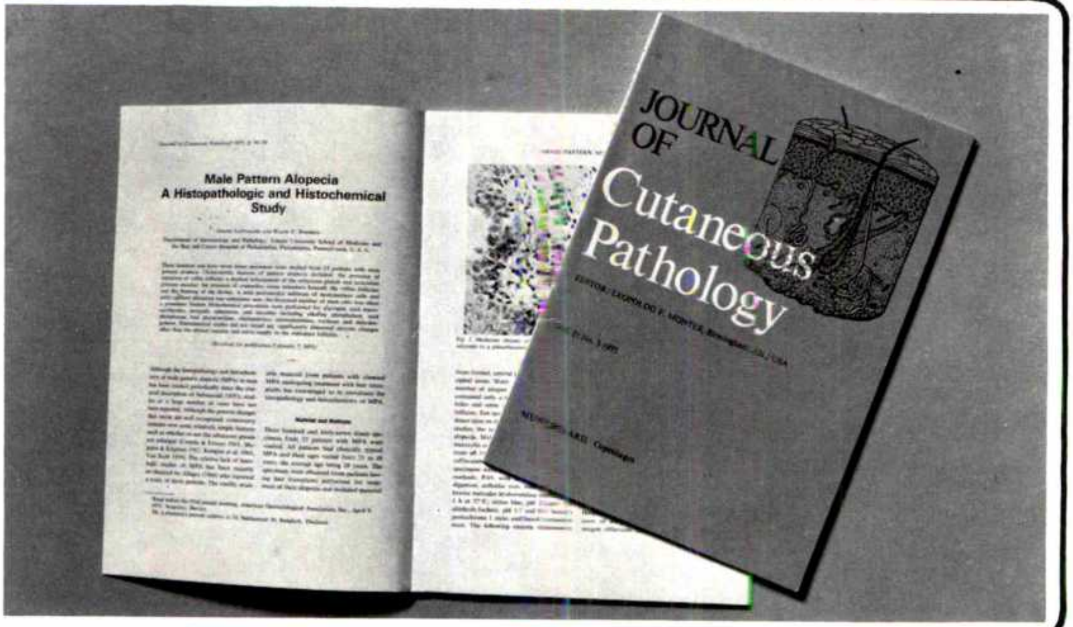

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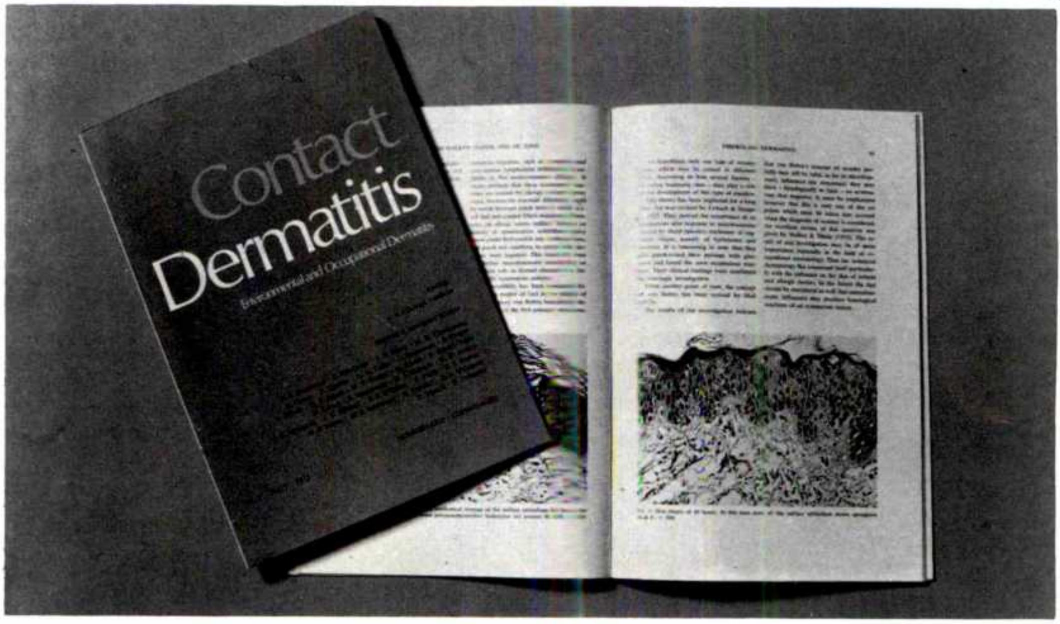

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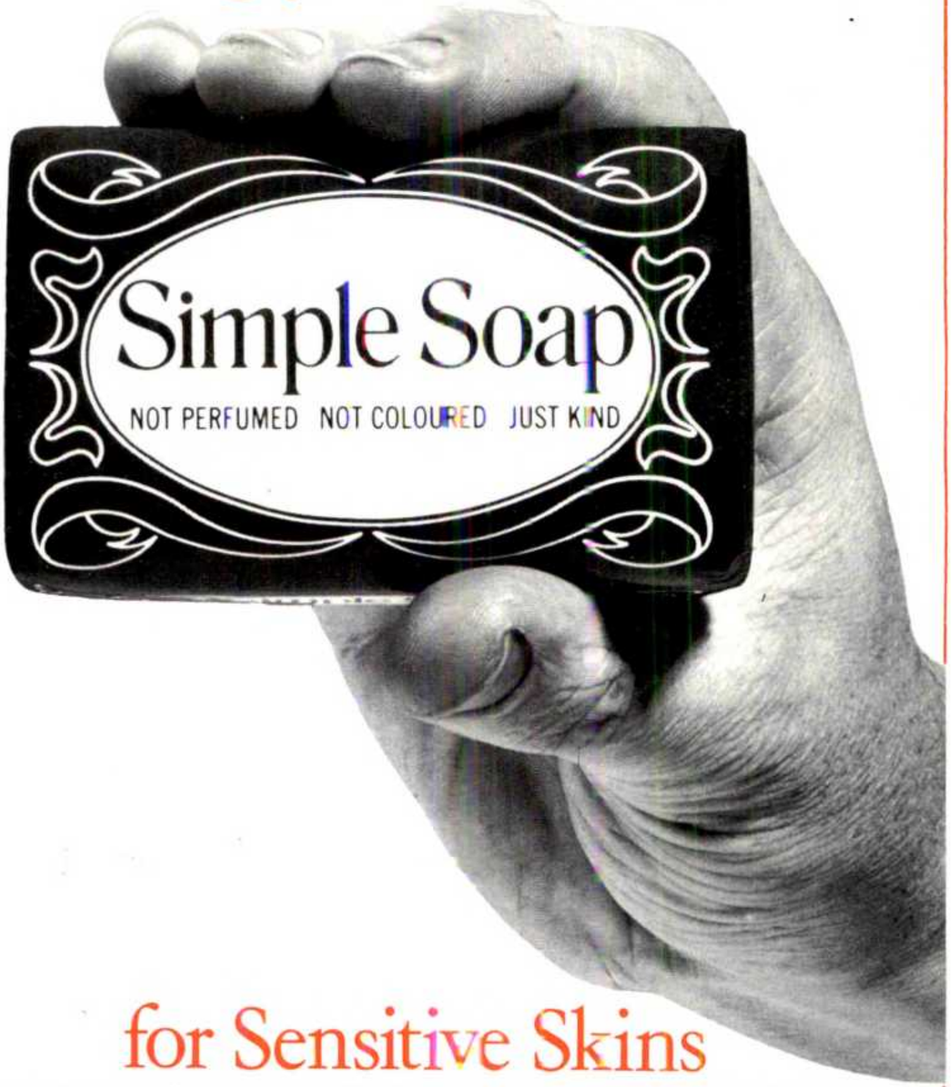

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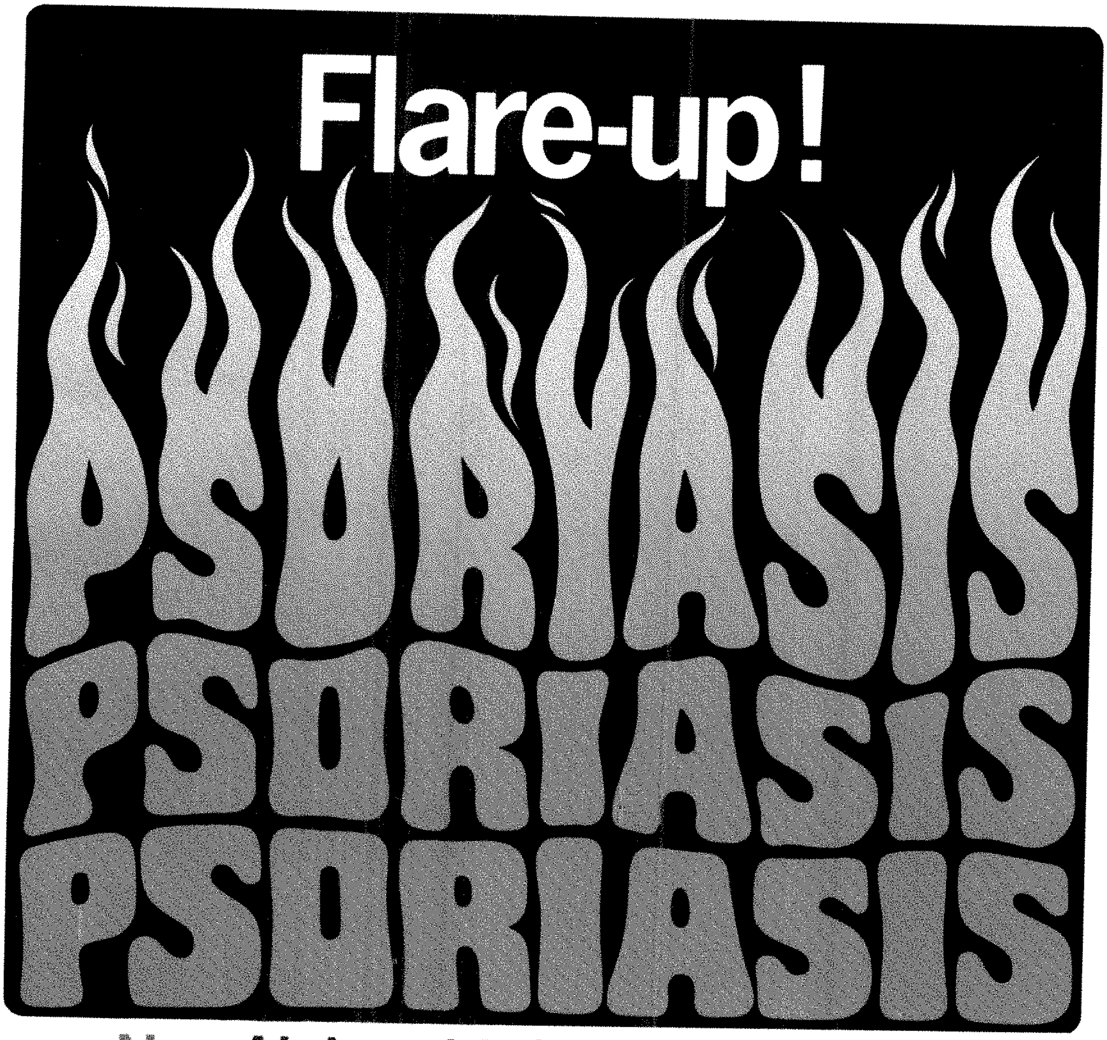

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British Journal of Dermatology (1977) **97**, 237.

A study of the *in vitro* metabolism of androgens by human scalp and pubic skin

J.B.HAY*

Department of Dermatology, University of Glasgow, Glasgow, Scotland

Accepted for publication 18 March 1977

SUMMARY

The metabolism of dehydroepiandrosterone, androstenedione and testosterone was compared *in vitro* in human scalp, forehead, pubic and axillary skin biopsies. Conversion of testosterone to the metabolite 5α-dihydrotestosterone, believed to be the active form of androgen, occurred in all tissues; however 17-oxosteroids such as androstenedione, 5α-androstanedione and androsterone were also formed from testosterone and were the major metabolites of scalp and forehead skin. While 17β-hydroxy steroid dehydrogenase activity was present in every skin sample, it was evident there were differences in the direction of operation of this enzyme in skin from different body sites. Axillary skin readily metabolised androstenedione and dehydroepiandrosterone to active 17β-hydroxy steroids such as testosterone and 5α-dihydrotestosterone, but these compounds were minor metabolites of androstenedione and dehydroepiandrosterone in forehead and scalp skin despite their activity in 17β-oxidation of testosterone. Pubic skin was intermediate between axillary and scalp skin in its ability to form 17β-hydroxy products from androstenedione and dehydroepiandrosterone. It is suggested these patterns of metabolism may reflect differences in androgen sensitivity.

The biological action of testosterone on many androgen controlled organs is thought to be mediated by *in situ* formation of an active metabolite, 5α-dihydrotestosterone, which is later transported to the nuclear chromatin (Bruchovsky & Wilson, 1968a, b). Skin functions such as sebum secretion (Strauss & Pochi, 1963) and body hair growth are well known to be under androgen control. Testosterone and other plasma androgens, such as androstenedione and dehydroepiandrosterone are converted to 5α-dihydrotestosterone by human skin (Gomez & Hsia, 1968; Hodgins, 1971; Hay & Hodgins, 1973). While human facial and axillary skin converted these three plasma androgens to 5α-dihydrotestosterone the amount formed *in vitro* appeared to depend on the direction of action of the skin 17β-hydroxy steroid dehydrogenase (17β-HSD) activity (Hay & Hodgins, 1973). In facial skin 17β-oxidation predominated over 17β-reduction and the 17-oxo steroids, 5α-androstanedione and androsterone were the main products rather than 5α-dihydrotestosterone, while in axillary skin 5α-dihydrotestosterone and other 17β-hydroxy steroids were major metabolites of all three plasma androgens. It was suggested that the metabolic pathway in axillary skin might be part of a mechanism allowing female androgen levels to stimulate axillary hair growth. It was decided to make similar studies of skin from other body sites, namely the pubic and scalp areas. The pubic area was chosen for comparison with the axilla because hair growth in both occurs early in puberty in both males and females and presents similarities in hormonal control (Hamilton, 1951; Rook, 1965). The scalp provides an area where hair growth is not markedly stimulated by androgens and where androgen dependent hair loss occurs in male pattern alopecia (Hamilton, 1942). A preliminary report of this work has already appeared (Hay, 1975).

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* Present address: University Department of Dermatology, Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP.

MATERIALS AND METHODS

Tissues

Pubic skin specimens were obtained from three women undergoing laparotomy. Scalp skin samples were obtained from a 29-year-old female and from three bald men aged 65, 60 and 50 during neurosurgery. These scalp specimens were from the frontal, nape, temporal and anterior vertex regions of the scalp respectively. Normal skin was also obtained from the axillas of two women aged 32 and 61 years and from the foreheads of two men aged 51 and 62 years during plastic surgery. A sample of frontal region scalp skin was also taken from this 62 year old bald male. A histological examination was carried out on the pubic and male scalp skins. The pubic skins contained terminal hairs and very small sebaceous glands. The scalp skin specimens from the bald males aged 50 and 62 years were from the bald region and appeared histologically typical of male pattern alopecia showing no evidence of other pathological loss of hair. Vellus type hair follicles were seen in these two samples; the skin from the other two males was from non-bald areas and contained terminal hairs. All four male scalp skins had well developed sebaceous glands, but only the specimen from the 50 year old male appeared to have the enlarged sebaceous glands sometimes found in bald scalp (Ellis, 1958). The biopsies were kept on ice till incubation (within 45 min of excision).

Radioactive steroids

[7α-^{3}H] testosterone (1·51 Ci/mmol), [7α-^{3}H] androstenedione (5·88 Ci/mmol) and [7α-^{3}H] dehydroepiandrosterone (0·50 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England. Before use they were purified by thin-layer chromatography on silica gel (Merck HF 254+366), using chloroform : acetone (185: 15, v/v) as solvent. Control experiments showed these purifications to be satisfactory.

Incubations

The skin was freed from subcutaneous fat, weighed and cut transversely into 0·5 mm thick slices with razor blades. The slices were incubated in 1 ml of Krebs improved Ringer 1 medium (Krebs, 1950), containing 3·5 nmol (1 μCi) radioactive steroid dissolved with the aid of 5 μl propane-1, 2-diol. The mixtures were incubated for 2 h at 37°C with shaking under O_2/CO_2 95:5. The reactions were stopped by the addition of 5 ml of acetone and 250 μg each of the appropriate unlabelled carrier steroids in ethanol.

Extraction and purification of steroids

Steroids were extracted from the tissue and medium, then purified by paper and thin layer chromatography as previously described (Hodgins, 1971; Hay & Hodgins, 1974).

Isolation and determination of steroids

Samples were removed from each purified steroid for estimation of radioactivity and carrier recovered (Hodgins, 1971; Hay & Hodgins, 1974). A further sample was taken for determination of the radiochemical purity by recrystallization to constant specific activity; 10 mg carrier were used and crystals were weighed on a Cahn Electrobalance (Cahn Instrument Co., Paramount, California, U.S.A.). No attempt was made to separate the components of the androstenediol-androstanediol fraction chromatographically but aliquots were taken for crystallization with the following carriers: 5-androstene-3β, 17β-diol, 5α-androstane-3β, 17β-diol and 5α-androstane-3α, 17β-diol, to give some indication of the composition of this fraction. 5α-Androstane-3α,- 17β-diol does not appear to cocrystallize with either 5-androstene-3β, 17β-diol or 5α-androstane-3β, 17β-diol. 5-Androstene-3β, 17β-diol and 5α-androstane-3β, 17β-diol show a tendency to cocrystallize but can be distinguished by further crystallization of their oxidation products. During oxidation with chromic acid (Bush 1961) [7α-^{3}H]5α-androstane-3β, 17β-diol is converted to [7α-^{3}H]5α-androstanedione while [7α-^{3}H]5-androstene-3β, 17β-diol is converted to 4-androstene-3,6, 17-trione with loss of 7α-^{3}H (Pierrepoint *et al.*, 1967).

RESULTS

The metabolic products obtained on incubating testosterone or androstenedione or dehydroepiandrosterone with slices of human scalp, pubic, forehead or axillary skin in Krebs improved Ringer 1 medium are shown in Tables 1–3. The results are expressed as per cent conversion of substrate in 2 h by 100 mg tissue and except for the polar neutral steroid fraction are corrected for losses which occurred during isolation of the steroids and are based on constant specific activities some of which are shown in Table 4. Fig. 1 summarizes the pathways of C_{19}-steroid metabolism in skin.

Under the conditions of incubation, more 5α-dihydrotestosterone was formed from testosterone than from other substrates. The metabolism of testosterone showed in all cases a marked tendency towards the formation of 17-oxo steroids (mainly androstenedione, 5α-androstanedione and androsterone), so that under the conditions used 17-oxo steroid accumulation exceeded that of the 17β-hydroxy steroids (5α-dihydrotestosterone and 5α-androstanediols). The highest ratios of 17β-hydroxy steroid/17-oxo steroid metabolites occurred in incubations of testosterone with axillary skin and the lowest with scalp and forehead skin.

The main products of androstenedione metabolism were 5α-androstanedione and androsterone. Metabolism of androstenedione to 17β-hydroxy steroids (testosterone, 5α-dihydrotestosterone and 5α-androstanediols) was highest with axillary skin and lowest with scalp and forehead skin.

Dehydroepiandrosterone was metabolized in all incubations to androstenedione and 5α-steroids; this activity was least prominent in scalp skin from the twenty-nine year old female and in pubic skin biopsies. Reduction of dehydroepiandrosterone to 17β-hydroxy steroids occurred to a greater extent with pubic and axillary skin than with scalp and face skin. In many dehydroepiandrosterone incubations 5-androstene-3β, 17β-diol was the main or only 17β-reduced product.

In every incubation unidentified radioactivity, probably the result of hydroxylase activity, was found in the neutral steroid fraction more polar than androstanediol. Dehydroepiandrosterone sulphate, 5α-androstane-3β, 17β-diol, aetiocholanolone and epiandrosterone were detected as minor metabolites in some incubations, but 5β-androstanedione and epitestosterone were not formed in amounts sufficient for identification.

Scalp skin from the sixty year old male was more active in testosterone metabolism than other scalp specimens, and while it gave a greater yield of 5α-dihydrotestosterone the ratio of metabolites produced was not much altered compared to other scalp specimens. This greater activity in testosterone metabolism did not extend to androstenedione or dehydroepiandrosterone metabolism. Testosterone metabolism in temporal skin from the 51-year-old male was also rather more extensive than the other facial skin specimens studied, but here too the product ratio was not grossly altered. In this case androstenedione and dehydroepiandrosterone metabolism were also somewhat raised. Forehead skin

TABLE 1. The metabolism of testosterone by scalp, forehead, axillary and pubic skin

	Scalp skin					Forehead skin		Axillary skin		Pubic skin		
Weight of tissue (mg)	153·1	134·6	59·4	108·6	82·6	85·2	70·5	131·7	20·7	59·5	78·1	46·0
Age of patient (years)	29	50	60	65	62	62	51	32	61	36	46	24
Sex of patient	F	M	M	M	M	M	M	F	F	F	F	F
Site and state of scalp	S, H	A, B	T, B	N, H	S, B							
Product												
Androstenedione	5·6	5·9	16·0	8·1	6·6	8·4	19·0	4·2	8·0	9·2	6·5	4·4
5α-Androstanedione	3·4	4·2	26·0	5·6	3·4	4·2	17·0	7·0	7·0	5·5	6·3	6·4
Androsterone	1·4	2·8	13·0	3·8	3·1	2·6	24·0	11·0	3·5	3·5	4·4	5·4
Epiandrosterone	0·18	0·16	1·0	0·38	0·18	0·16	0·23	0·65	0·28	0	0	0
Aetiocholanolone	0·07	0·09	0·06	0	0·12	0	0	0	0	0	0	0
5α-Dihydrotestosterone	1·8	2·4	5·2	2·6	2·1	1·4	7·4	7·8	7·6	5·2	7·5	8·1
5α-Androstane-3α,17β-diol	0·42	0·74	1·7	0·85	0·66	0·29	3·7	10·0	3·8	1·7	2·5	3·3
5α-Androstane-3β,17β-diol	0	0·09	0	0·12	0	0	0·28	0·96	0	0·16	0	0·80
Polar neutral steroid	0·50	1·0	5·0	1·9	1·2	1·1	4·1	6·5	3·3	1·3	1·7	2·2

Results are expressed as percentage conversion of substrate by 100 mg tissue in 2 h at 37°C. In Tables 1–3 M and F refer to male and female, S, A, T and N indicate the use of frontal, anterior vertex, temporal and nape scalp skin, and B and H indicate the skin to be bald or non-bald (hairy) respectively.

TABLE 2. The metabolism of androstenedione by scalp, forehead axillary and pubic skin

	Scalp skin					Forehead skin		Axillary skin		Pubic skin		
Weight of tissue (mg)	130·8	122·5	81·0	106·5	100·1	102·0	71·6	107·1	22·5	33·4	70·4	92·1
Age of patient (years)	29	50	60	65	62	62	51	32	61	24	36	46
Sex of patient	F	M	M	M	M	M	M	F	F	F	F	F
Site and state of scalp	S, H	A, B	T, B	N, H	S, B							
Product												
5α-Androstanedione	11·0	11·0	12·0	7·1	9·0	6·4	36·0	13·0	41·0	23·0	19·0	11·0
Androsterone	5·3	7·0	8·7	3·8	4·2	3·3	42·0	26·0	23·0	18·0	11·0	17·0
Epiandrosterone	0·46	0·37	0·68	0·92	0·26	0·28	1·2	0·97	2·5	2·4	0·68	0·47
Aetiocholanolone	0·29	0	0·56	0	0	0·45	0	0	0	0·90	0·49	0·79
Testosterone	0·33	0·36	0·53	0·47	0·22	0·32	0·50	0·58	1·5	2·3	1·3	1·4
5α-Dihydrotestosterone	0·29	0·33	0·28	0·27	0·15	0·15	0·75	6·3	5·1	0·72	0·46	1·4
5α-Androstane-3α,17β-diol	0·15	0·20	0·21	0·21	0	0	1·5	13·0	6·6	0	0·38	2·2
5α-Androstane-3β,17β-diol	0	0	0	0	0	0	0	0·85	0·56	0	0	0
Polar neutral steroid	1·5	1·1	2·3	1·4	1·2	1·4	5·2	9·1	16·0	2·5	1·6	2·5

Results are expressed as percentage conversion of substrate by 100 mg tissue in 2 h at 37°C.

TABLE 3. The metabolism of dehydroepiandrosterone by scalp, forehead, axillary and pubic skin

	Scalp skin					Forehead skin		Axillary skin	Pubic skin		
Weight of tissue (mg)	162·4	103·8	72·0	121·0	112·6	64·1	67·3	126·3	43·0	78·8	102·5
Age of patient (years)	29	50	60	65	62	62	51	32	24	36	46
Sex of patient	F	M	M	M	M	M	M	F	F	F	F
Site and state of scalp	S,H	A,B	T,B	N,H	S,B						
Product											
Androstenedione	0·63	1·0	1·4	0·92	1·3	0·60	1·2	0·91	0·84	0·38	0·42
5α-Androstanedione	0·81	3·9	5·4	2·7	2·9	2·2	5·3	4·5	0·21	0·66	1·6
Androsterone	0·60	3·3	3·7	2·7	1·9	1·8	6·1	6·8	0	0·21	1·2
Epiandrosterone	0	0	0	0	0·33	0	0·39	0·97	0	0	0
Aetiocholanolone	0	0	0	0	0	0	0	0	0	0	0
Dehydroepiandrosterone sulphate	0·11	0·17	0·40	0·50	0·10	0	0	0·12	0·91	0	0
Testosterone	0·07	0·12	0	0·21	0·13	0	0	0·47	0	0	0
5α-Dihydrotestosterone	0·08	0	0	0	0·20	0·18	0	3·7	0	0	0
5α-Androstane-3α,17β-diol	0	0	0	0	0	0	0	7·5	0	0	0
5α-Androstane-3β,17β-diol	0	0	0	0	0	0	0	0	0	0	0
5-Androstene-3β,17β-diol	0·30	0·32	0·46	0·39	0·20	0·29	1·1	5·3	6·9	3·5	2·2
Polar neutral steroid	3·1	5·9	11·0	6·0	7·1	6·8	7·5	17·0	21·0	5·3	5·4

Results are expressed as percentage conversion of substrate by 100 mg tissue in 2 h at 37°C.

TABLE 4. Examples of constant specific activities obtained on recrystallization (d.p.m./mg)

	1	2	3	Skin tissue	Patient's age
Testosterone	1211	1282	1267	Pubic	46 (A)
Testosterone	315	325	312	Scalp	65 (D)
5α-Dihydrotestosterone	7232	7188	7167	Scalp	50 (T)
5α-Dihydrotestosterone	4895	4930	4982	Pubic	46 (T)
5α-Dihydrotestosterone	191	200	204	Pubic	36 (A)
5α-Dihydrotestosterone	300	319	324	Scalp	29 (A)
5α-Androstane-3α,17β-diol	1342	1251	1326	Scalp	50 (T)
5α-Androstane-3α,17β-diol	1146	1124	1111	Pubic	24 (T)
5α-Androstane-3α,17β-diol	170	159	165	Scalp	50 (A)
5α-Androstane-3α,17β-diol	199	194	184	Pubic	36 (A)
5α-Androstane-3α,17β-diol	40879	40540	39845	Axilla	32 (T)
5α-Androstane-3α,17β-diol	37894	39864	40216	Axilla	32 (A)
5α-Androstane-3α,17β-diol	6192	6090	6141	Axilla	32 (D)
5α-Androstane-3β,17β-diol	272	269	283	Pubic	24 (T)
5α-Androstane-3β,17β-diol	143	152	144	Scalp	50 (T)
5α-Androstane-3β,17β-diol	3191	3268	3178	Axilla	32 (T)
5α-Androstane-3β,17β-diol	2031	1951	2105	Axilla	32 (A)
5-Androstene-3β,17β-diol	271	257	263	Scalp	29 (D)
5-Androstene-3β,17β-diol	419	410	392	Pubic	24 (D)
5-Androstene-3β,17β-diol	8319	7906	8543	Axilla	32 (D)
Androsterone	19721	19500	19791	Scalp	60 (T)
Androsterone	12088	11842	12070	Pubic	24 (A)
5α-Androstanedione	5218	5328	5349	Scalp	65 (A)
5α-Androstanedione	9296	9482	9023	Pubic	78 (D)
Androstenedione	3246	3193	3237	Pubic	24 (T)
Androstenedione	2089	2095	2160	Scalp	29 (D)

T, A and D refer to the substrates testosterone, androstenedione and dehydroepiandrosterone respectively.

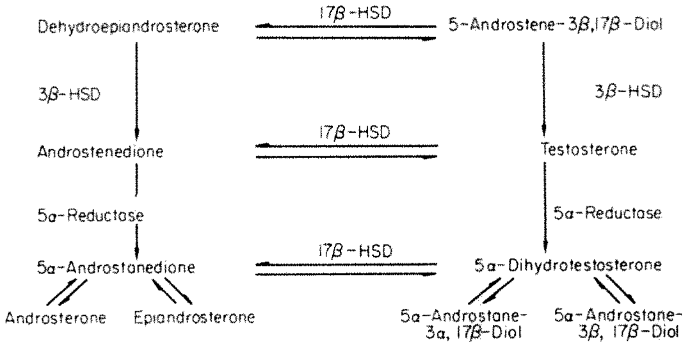

FIGURE 1. Possible pathways of C19 steroid metabolism in human skin, 17-oxo steroids are on the left and 17β-hydroxy steroids on the right. In addition to the reactions shown dehydroepiandrosterone, androstenedione and testosterone are converted to hydroxylated metabolites. Dehydroepiandrosterone sulphate and aetiocholanolone may be formed from dehydroepiandrosterone and androstenedione respectively.

from the 62-year-old male and scalp specimens other than the 60-year-old male seemed less active in 5α-reduction than the axillary and pubic skin specimens.

DISCUSSION

The conversion of androgens to 5α-dihydrotestosterone is thought to be necessary for their action on the target organ (Bruchovsky & Wilson, 1968a, b). The enzyme Δ^4-3-oxo C19 steroid 5α-reductase (5α-reductase) is frequently assumed to play a controlling role in this metabolism. While this may be true for testosterone substrate, it is clear from Fig. 1 that formation of 5α-dihydrotestosterone from androstenedione or dehydroepiandrosterone substrates must also involve 17β-HSD activity. Experiments using several androgen substrates (Hay & Hodgins, 1973) showed that while two axilla skin specimens and four facial skin specimens were active in oxidizing testosterone to 17-oxo steroids, only the axillary specimens showed any great activity in converting androstenedione and dehydroepiandrosterone to 5α-dihydrotestosterone indicating that 17β-HSD activity itself may vary in the direction of its operation in human skin. In the present case two further axilla and forehead skin specimens have conformed to this pattern. These studies indicate that this is most likely a body site difference not directly dependent on age or sex of patient.

A previous study of androgen metabolism in scalp was confined to testosterone substrate (Bingham & Shaw, 1973). The products of testosterone, androstenedione and dehydroepiandrosterone metabolism in scalp skin from five patients were found during the present work to be qualitatively and proportionately similar to those expected for forehead skin, the 17β-HSD activity being predominately oxidative. Little qualitative difference in the ratio of products was noted between the five scalp specimens despite the use of bald skin in two cases and differences in site of origin.

The predominantly oxidative activity of 17β-HSD in human scalp, forehead, shoulder and thigh skin (Hay & Hodgins, 1974) may indicate this situation to be widespread. However increased conversion of androstenedione and dehydroepiandrosterone substrates to 17β-hydroxy steroids was noted in pubic skin compared to scalp and forehead skin though this process was less active than in axillary skin. It was previously suggested that the difference between axillary and forehead skin was controlled by variations in 17β-HSD activity and not 5α-reductase (Hay & Hodgins, 1973). However, most of the metabolites from which this was deduced are products of both enzymes and in the present case the 5α-reductase activity of pubic skin appears higher than scalp. Testosterone and 5-androstene-3β, 17β-diol are direct 17β-reduction products of androstenedione and dehydroepiandrosterone substrates respectively and the formation of these two compounds was higher with pubic skin than scalp or forehead skin. It is then likely that both increased 17β-reduction and raised 5α-reductase contribute to the greater formation of active 17β-hydroxy steroids in pubic skin. The metabolism of testosterone in human suprapubic and linea alba skin has been described previously (Jenkins & Ash, 1973; Thomas & Oake, 1974). Studies of dehydroepiandrosterone metabolism in linea alba skin showed increased 17β-hydroxy steroid synthesis in hirsutism (Thomas & Oake, 1974); however the 17β-hydroxy steroids isolated by those authors were products of both 17β-HSD and 3β-hydroxy steroid dehydrogenase 4–5 isomerase (3β-HSD) activities (the latter enzyme is localized mainly in sebaceous glands and sebaceous activity is raised in hirsutism) (Baillie, Calman & Milne, 1965; Burton *et al.*, 1972).

Although the present incubation conditions depart from the physiological, the use of lower substrate concentrations does not alter the direction of action of facial or axillary skin 17β-HSD activity (unpublished observations, Hodgins & Hay). It is therefore possible the greater formation of 5α-dihydrotestosterone and other 17β-hydroxy steroids by axillary and pubic skin reflects an increased sensitivity to androgens at these sites. This correlates with the early growth of axillary and pubic hair in puberty in both males and females. Increased formation of 17β-hydroxy steroids from dehydroepiandrosterone might indicate a sensitivity of these areas to adrenal androgens. This appears to be supported by the limited growth of hair in these regions in prepubertal Addison's disease (Rook, 1965) and reported loss of hair in some adult cases of Addisonism (Allen, 1954). Children with precocious hair growth in these regions show elevated plasma levels of dehydroepiandrosterone and dehydroepiandrosterone sulphate derived from the adrenals (the rise in these two plasma androgens occurs considerably before that of testosterone during puberty) (Rosenfield, 1971; Korth-Schultz *et al.*, 1975; Sizonenko, Paunier & Carmignac, 1975).

The low activity of scalp and forehead skin in forming 17β-hydroxy steroids from dehydroepiandrosterone does not argue for a large role for adrenal androgens in these regions. Acne vulgaris, an androgen dependent syndrome, may however respond to adrenal suppression (Potter, 1971), and plasma dehydroepiandrosterone sulphate levels are often raised in women with acne (Walker *et al.*, 1975). However acne develops later in puberty than axillary hair growth and may depend on higher plasma steroid levels for the provision of 5α-dihydrotestosterone. In addition, other plasma steroids levels are altered in acne (Forstrom *et al.*, 1974; Rose *et al.*, 1976) so it is not yet possible to ascribe the action of the adrenal solely to dehydroepiandrosterone sulphate.

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Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex

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SUMMARY

Androgenetic alopecia in the female occurs much more frequently than is generally believed. The condition is still considered infrequent, for it differs, in its clinical picture and in the sequence of events leading to it, from common baldness in men. To facilitate an early diagnosis (desirable in view of the therapeutic possibilities by means of antiandrogens) a classification of the stages of the common form (female type) of androgenetic alopecia in women is presented. The exceptionally observed male type of androgenetic alopecia can be classified according to Hamilton or to the modification of this classification proposed by Ebling & Rook.

The occurrence of androgenetic alopecia (common baldness) in both sexes is uncontested (Sabouraud, 1902; McCarthy, 1940; Maguire & Kligman, 1963; Ludwig, 1964a; Rook, 1965; Montagna & Parakkal, 1974). Although Behrman (1952) pointed out that androgenetic alopecia in the female sex ('female pattern baldness') is of more frequent occurrence than generally believed (a statement that has been confirmed since by Binazzi & Wierdis (1960); Barman, Pecoraro & Astore (1962); Smith & Wells (1964); Ludwig (1964b); Vadász & Debreczeni (1967); Goldschmidt (1969)), it is still considered infrequent or even exceptional (Pillsbury, Shelley & Kligman, 1956). The main reason for this erroneous belief is that many cases of sparseness or diffuse thinning of the hair are rather meaninglessly diagnosed as 'diffuse alopecia'. This misinterpretation often occurs because many dermatologists are unaware that the clinical picture of androgenetic alopecia in the female and the sequence of events leading to it differ from those observed in men.

Treatment with antiandrogens during early stages can arrest or at least retard the progress of androgenetic alopecia to a stage where correction with a wig would be required (Neale, Krebs & Bettendorf, 1971; Winkler & Schäfer, 1972; Ludwig & Zaun, 1973). Therefore, early and correct diagnosis is highly desirable. Accordingly the objective of this paper is to facilitate the diagnosis by a classification of the consecutive stages of androgenetic alopecia in women, comparable to that given by Hamilton (1951) for the common baldness in men. The following introductory remarks may contribute to a better understanding of the proposed classification.

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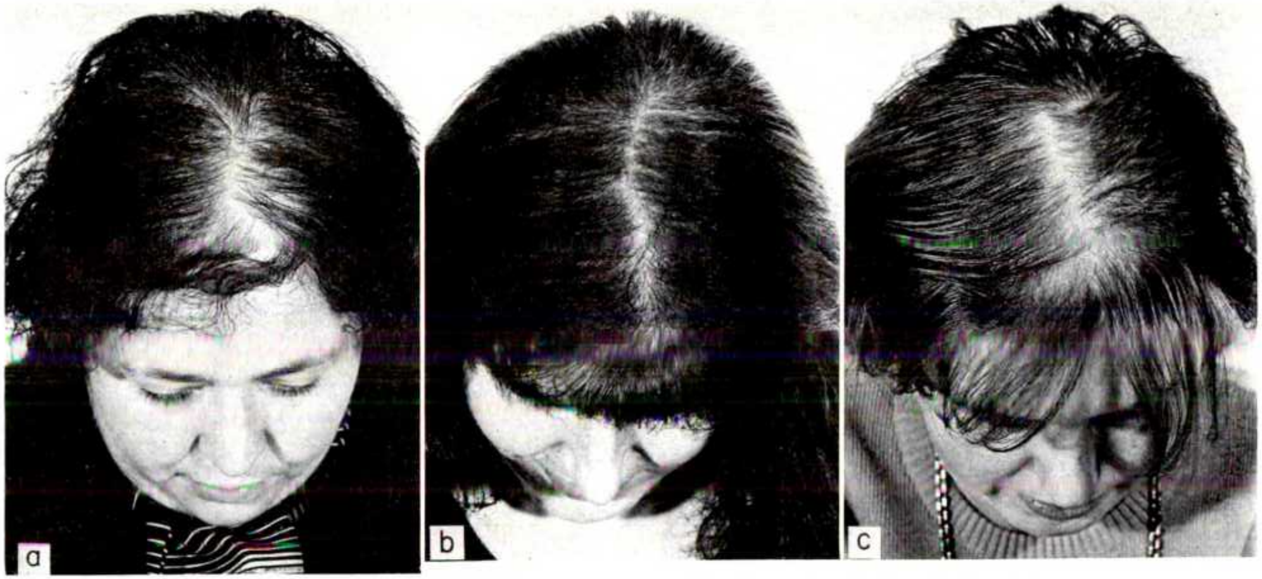


FIGURE 1. (a–c). Perceptible thinning of the hair on the crown with a well preserved frontal fringe. Early stage of the female type of androgenetic alopecia.

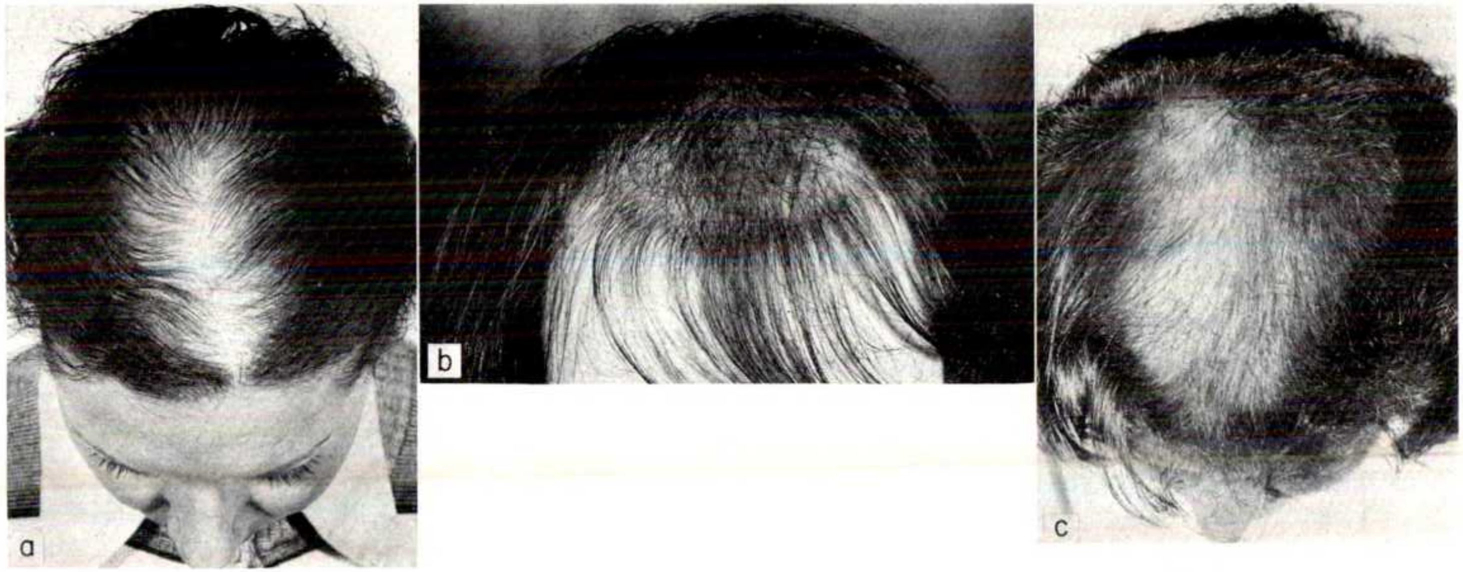


FIGURE 2 (a–c). Pronounced thinning of the hair on the crown still showing a well preserved frontal fringe. Advanced stage of the female type of androgenetic alopecia.

(Fig. 2b reproduced from Zaun, H. (1963) *Zeitschrift für Haut und Geschlechtskrankheiten*, **35**, 35.)

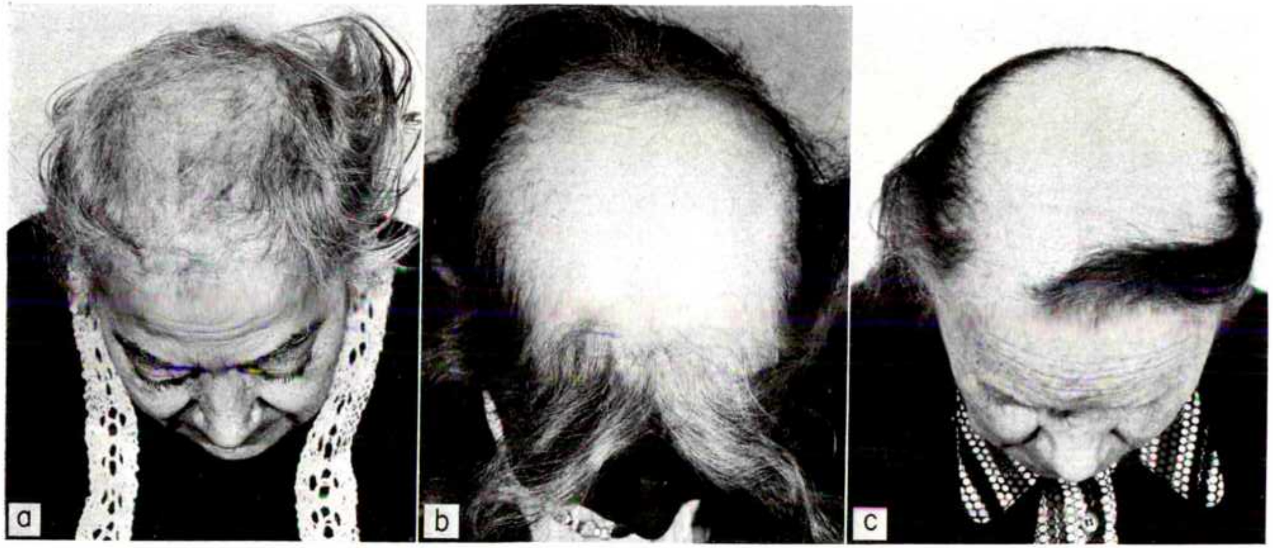


FIGURE 3 (a–c). Total or almost total denudation of the crown frontally still limited by a fringe of hair. Facultative final stage of the female type of androgenetic alopecia.

CLINICAL PICTURE

The balding process in the female starts with a uniform rarefaction of the hair on the crown. The resulting oval shaped area of rarefied hair is surrounded by a circular band of normally dense hair of variable breadth. Frontally the fringe is narrow (1–3 cm), laterally in the temporo–parietal region about 4–5 cm wide. On the back of the head the fully haired occiput is separated from the area of rarefied hair by a semicircular line lying between the vertex and the occipital protuberance. Thus the shape of the area in the female closely resembles that in far advanced male baldness, differing from it only by the preserved frontal fringe.

Within this oval shaped area on the crown the hair is definitely rarefied. Besides inconspicuous hairs of normal length there is a variable percentage of hairs which are thinner, shorter, and occasionally less pigmented. A usually well preserved fringe of hair along the frontal hair line is quite characteristic. (Fig. 1a–c).

With advancing age, the rarefaction on the crown within the aforementioned area becomes more pronounced and the number of thinner and shorter hairs increases. Camouflage of the denudation by special hair styles is no longer possible (Fig. 2a–c). In some women, usually not before the menopause, the crown may become literally bald. Contrary to what happens in men, a fringe of hair along the frontal hair line persists. (Fig. 3a–c). This development is the commonly observed one and represents what can be named the *female type of androgenetic alopecia*

Apart from this 'female type' androgenetic alopecia in females can in exceptional cases follow a course similar to that seen in men. In such cases the process starts with deep fronto-temporal recessions and the following stages are identical to those defined by Hamilton (1951) (Figs 5–7).

In postmenopausal women occasionally one can observe a special form of alopecia. Here the diffuse thinning of the hair is limited to an orbicular zone around the vertex (skull-cap alopecia). The condition is possibly a variant of androgenetic alopecia. The preceding stages of this form are, however, not known, nor were testosterone excretion studies performed in such cases.

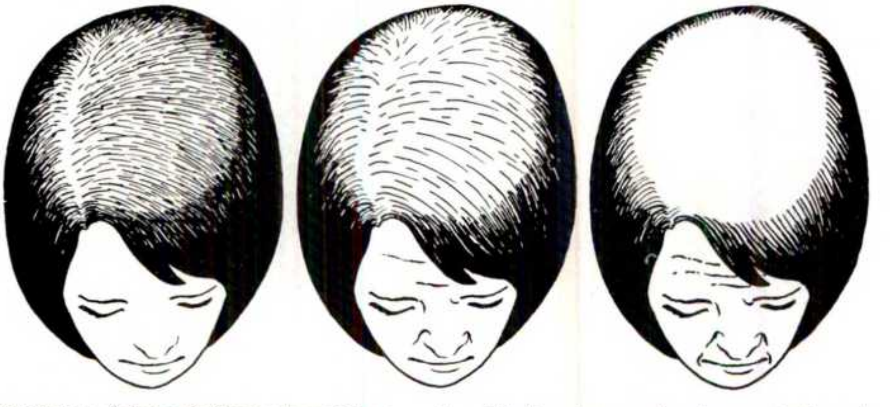
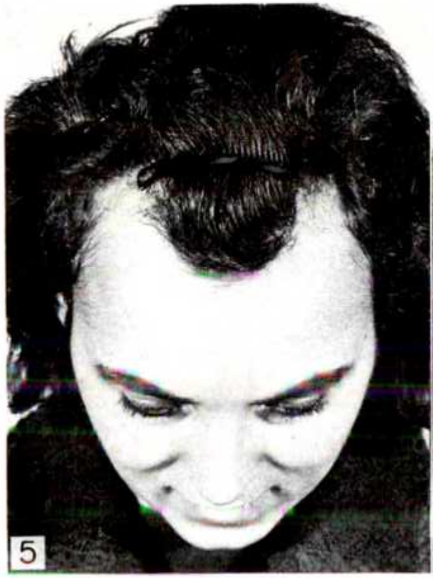
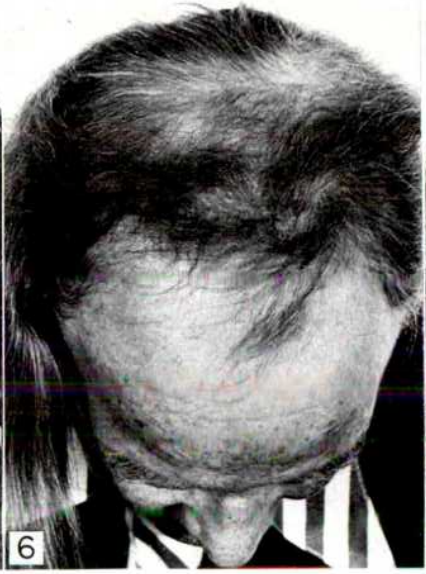


FIGURE 4. Schematic illustration of the 3 grades of the female type of androgenetic alopecia as shown in Figs 1–3.

CLASSIFICATION

On the basis of 468 cases observed during a period of several years the stages of development of androgenetic alopecia in the female may be classified as follows:





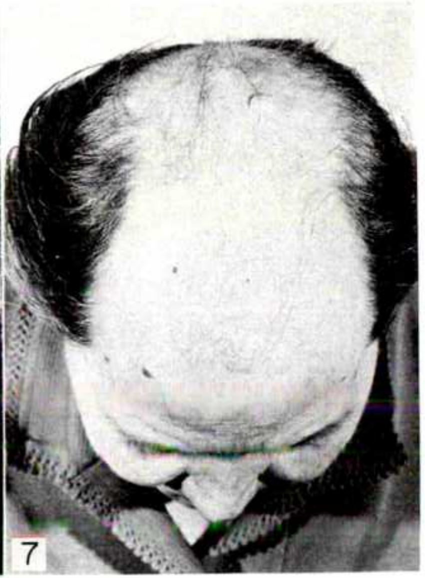


FIGURE 5. Deep fronto-temporal recessions in a 24 year-old female patient with accompanying hirsutism. Male type of androgenetic alopecia corresponding to Hamilton type IV (I in the modification of Ebling–Rook).

FIGURE 6. Male type of androgenetic alopecia in a 66 year-old female patient treated for years with testosterone-containing injections. Corresponding to Hamilton type VII (IV in the modification of Ebling–Rook).

FIGURE 7. Hippocratic baldness in a 57 year-old female patient with extremely high urinary testosterone excretion. Corresponding to Hamilton type VIII (V in the modification of Ebling–Rook).

Grade I. Perceptible thinning of the hair on the crown, limited in the front by a line situated 1–3 cm behind the frontal hair line (Fig. 1a–c and 4a).

Grade II. Pronounced rarefaction of the hair on the crown within the area seen in Grade I (Fig. 2a–c and 4b).

Grade III. Full baldness (total denudation) within the area seen in Grades I and II (Fig. 3a–c and 4c).

These grades I–III which represent stages of the female type of androgenetic alopecia are schematically illustrated in Fig. 4a–c.

The stages of development of the rather exceptional 'male type' (Figs 5–7) may be classified according to Hamilton (1951) or to Ebling & Rook (1972), who in their modification of Hamilton's original classification distinguish 5 types of common baldness in the male.

For instance Fig. 5 can be classified equal to Hamilton IV (or Ebling and Rook I), Fig. 6 equal to Hamilton VII (or Ebling and Rook IV) and Fig. 7 equal to Hamilton VIII (or Ebling & Rook V).

DISCUSSION

Sex determined differences in the aspect, structure and function of various human organs are well known. However, the reasons for different appearances of androgenetic alopecia in the male and the female and the occurrence of two different types of the same condition in the female are still not fully understood. Based upon urinary testosterone excretion studies (Apostolakis, Ludwig & Voigt, 1965; Pierard, Kint & de Backer, 1968) the view has been expressed (Ludwig, 1968) that there is a relationship between the degree of androgenic stimulation and the type of androgenetic alopecia which develops in genetically predisposed females. Thus moderately increased levels of circulating androgens appear to be correlated with the female type, while women with testosterone levels comparable with those in normal men develop the male type of baldness, indistinguishable from that occurring in men. In agreement with this concept the patient shown in Fig. 5 had besides her deep fronto-parietal recessions also a pronounced hirsutism. The female patient of 66 years of age (Fig. 6) has been treated for years with injections of high doses of testosterone. The patient shown in Fig. 7 had a urinary excretion of testosterone far exceeding all values found in normal young men. Further comparative endocrinological studies of females with the male and female type of androgenetic alopecia are required to corroborate the validity of this concept.

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Vitiligo: a new classification and therapy

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SUMMARY

Local injection of physostigmine revealed that dermatomally distributed vitiligo was associated with a dysfunction of the sympathetic nerves in the affected skin and that non-dermatomally distributed vitiligo was not. These observations led to the hypothesis that the primary disturbance of dermatomally distributed vitiligo lies in the sympathetic nerves of the affected area and that non-dermatomally distributed vitiligo has its primary disturbance in the melanocyte itself, where an autoimmune mechanism is suspect. Results of therapy supported this hypothesis by showing that topical corticosteroid is effective only in the latter, while the former reacts to oral nialamide.

It is proposed that non-dermatomally distributed vitiligo be referred to as Type A and dermatomally distributed vitiligo as Type B.

Previously, hypotheses concerning the pathogenesis of vitiligo and the reports supporting them have been confusing, and many have contradicted each other. None of these hypotheses has been able to explain all the phenomena observed in vitiligo and all have assumed or implied a single pathogenesis for the disease. This paper introduces a new hypothesis, based on a classification of vitiligo into two new types: Type A, non-dermatomally distributed; and Type B, dermatomally distributed, each having a separate pathogenesis. This hypothesis reasonably explains many previous observations, and is well supported both by a new nervous activity test, using physostigmine, which led to the formulation of the hypothesis, and by therapeutic experiments with topical corticosteroid and oral nialamide.

MATERIALS AND METHODS

120 male and female patients with vitiligo were subjected to the nervous activity test, detailed later, both in an affected area and in a symmetrical control area. They were then divided clinically into two groups according to type, those with dermatomally distributed vitiligo, Type B, and those with non-dermatomally distributed vitiligo, Type A. Ninety-five patients with vitiligo, 90 of whom displayed non-dermatomal distribution and 5 dermatomal distribution, were treated by the topical application of corticosteroid ointment or cream for 2–21 months. Nialamide was administered orally for 6–23 months to 32 patients with vitiligo ranging in age from 15 to 46.

Nervous activity test

Physostigmine, in conjunction with the Wada–Takagaki method of sweat detection (Wada & Takagaki, 1948), was used as an indication of nervous activity. Physostigmine injected intracutaneously inhibits the activity of cholinesterase, which inactivates the acetylcholine appearing at the sympathetic nerve endings of the eccrine sweat glands, thereby inducing perspiration from the eccrine sweat glands controlled by endogenous nervous activity. Iodine 3% in ethanol was applied to the vitiliginous area and to a symmetrical control area; after it had dried, a mixture of equal weights of starch and olive oil was applied to the areas. Physostigmine 0·05ml of 0·05% solution was then injected intracutaneously through the iodine and starch/olive oil membrane. After 30 min the sweat points in the area were revealed as black spots by the starch/iodide reaction.

The degree of sweating induced by the physostigmine was classified into 3 groups in comparison with the control site:

(1) Hypohidrotic group (Fig. 1); (2) Normohidrotic group (Fig. 2); (3) Hyperhidrotic group (similar to Fig. 1, with the control and specimen areas interchanged).

Treatment with topical corticosteroid

0·12% betamethasone-17-valerate, 0·01% fluocinolone acetonide or 0·1% triamcinolone acetonide cream or ointment was applied to the vitiliginous area 3 times a day. No other therapy was undertaken. Photographs were taken before and during the therapy. Patients were seen at 2–3 weekly intervals and were treated as out-patients.

Treatment with nialamide

On physical and laboratory examination, detailed later, all patients receiving this therapy were found to be healthy apart from their vitiligo. Nialamide, 150 mg (6 tablets) per day, was prescribed orally in 3 doses to each patient. It is a monoamine oxidase inhibitor which suppresses the metabolism of catecholamines at the sympathetic nerve endings. Photographs were used to follow the progress of the therapy. Patients were seen at 2 weekly intervals, and side effects were checked by physical examination. RBC, Hb, WBC and differential, liver function tests and urine tests were estimated before and every 3–4 months during the course of treatment.

RESULTS

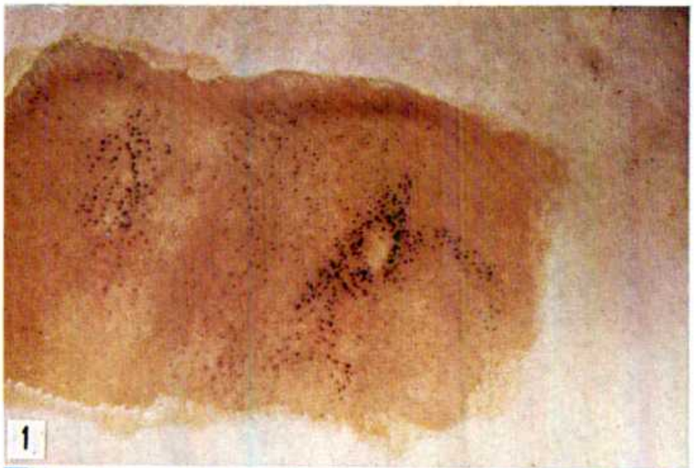
Nervous activity test

Of the 120 patients, 63 belonged in the normohidrotic group: 59 of them (93·7%) exhibited non-dermatomal distribution and 4 (6·3%) dermatomal distribution. Thirty-nine patients belonged in either the hypo- or hyperhidrotic groups: 34 of these (87·2%) exhibited dermatomal distribution and 5 (12·8%) non-dermatomal distribution.

Among the 34 dermatomally distributed patients, many who had been affected recently revealed hypohidrotic areas. While those with areas of long term duration, and where the margin of the affected area was hyperpigmented, revealed hyperhidrotic areas. Eighteen patients of this series could not be classified with certainty. Thus, there was significant association between dermatomally distributed vitiligo and a dyshidrotic state of the affected skin ($P < 0·01$). The results of this test are shown in Table 1.

Treatment with topical corticosteroid

As shown in Table 2, 95 patients received topical corticosteroid therapy for at least 2 months, and no improvement was found among the 5 patients with dermatomal distribution. Many of the patients with non-dermatomal distribution reacted well to the topical corticosteroid, especially those with vitiliginous areas of under 3 months duration. Differences in effectiveness were seen



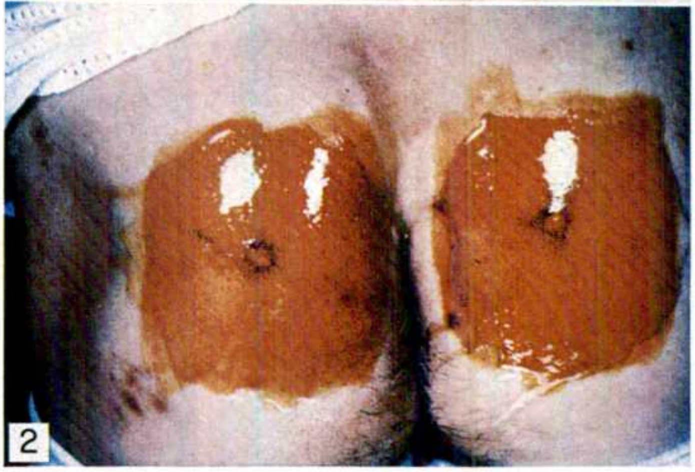


FIGURE 1. Nervous activity test: Hypohidrotic type. Sweat points in the affected area (left) are fewer than in the control area.

FIGURE 2. Nervous activity test: Normohidrotic type. Sweat points in the affected area (left) are almost equal in number to those in the control area.

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TABLE 1. The relationship between the results of the nervous activity test and the clinical distribution of the affected area in 120 vitiligo patients

	Dermatomal	Non-dermatomal
Dyshidrotic (hypo- or hyperhidrotic)	34	5
Normohidrotic	4	59
Unclassified	6	12

TABLE 2. The results of topical corticosteroid, with reference to the clinical distribution and duration time, in 95 vitiligo patients

Duration of vitiligo (years)	No. of treated cases	No. of improved cases	% improvement
Dermatomal			
<3/12	3	0	0
3–6/12	0	0	0
6–12/12	0	0	0
1–2	0	0	0
2–5	1	0	0
> 5	1	0	0
Total	5	0	0
Non-dermatomal			
< 3/12	18	17	94·4
3–6/12	14	9	64·3
6–12/12	13	11	84·6
1–2	18	16	88·9
2–5	10	6	60·0
> 5	17	15	88·2
Total	90	74	82·2

according to site. When the face and neck were affected by non-dermatomally distributed vitiligo, topical corticosteroid therapy was most promising and complete healing with only this method of treatment was not rare. Vitiligo on other parts of the body responded less well, and on the fingers the worst. The earliest repigmentation using this treatment was seen after 3 weeks. If improvement was not apparent after 3 months, the efficacy of continued corticosteroid treatment was doubtful. Improvement occurred in 82·2% of the 90 patients with non-dermatomal distribution, but in none of the 5 patients with dermatomal distribution. Partial improvement is included in this 82·2%.

Treatment with nialamide

The results of oral nialamide treatment are shown in Table 3. About half the patients with dermatomally distributed vitiligo were improved with nialamide, but none of the non-dermatomal cases. The degree of improvement was not so marked with this method, resulting in only partial reduction of the affected area.

TABLE 3. The results of oral nialamide, with reference to the clinical distribution and duration time, in 32 vitiligo patients

Duration of vitiligo (years)	No. of treated cases	No. of improved cases	% improvement
Dermatomal			
< 1	18	12	66·7
> 1	10	4	40
Total	28	16	55·6
Non-dermatomal			
< 1	2	0	0
> 1	2	0	0
Total	4	0	0

DISCUSSION

Of the various hypotheses of the pathogenesis of vitiligo Lerner (1972) selected 3, one related to autoimmunity, one concerning neurochemical factors and another suggesting the self-destruction of melanocytes. Copeman, Lewis & Bleehen (1973) also pointed out these three hypotheses, but many clinical and experimental observations cannot be explained adequately by any of them. Many authors have found an increased incidence of vitiligo associated with presumed autoimmune disorder, thyroid disease (Cunliffe *et al.*, 1968; Ochi & DeGroot, 1969), pernicious anaemia (Dawber, 1970; Grunnet *et al.*, 1970), diabetes mellitus (Dawber, 1968; Pettit, Landing & Guest, 1961). These presumed autoimmune diseases often appear together, and genetically determined predisposition to form organ specific antibodies (parietal cell antibodies) has been observed in these diseases, as well as in vitiligo (Bor, Feiwel & Chanarin, 1969). These observations strongly support an autoimmune mechanism in the pathogenesis of vitiligo and yet they remain circumstantial and the only report to show the existence of an antimelanin antibody in vitiligo (Langhof, Feuerstein & Schabinski, 1965) has not been supported by any other investigations. Moreover, the autoimmune hypothesis cannot explain many phenomena concerning the dysfunction of the autonomic nervous system in the vitiliginous area. Many authors have reported data suggesting disturbance of the autonomic nerves in the vitiliginous skin, but they have not been in agreement as to the site of the disturbance. One report (Chanco-Turner & Lerner, 1965) mentioned an increase of skin temperature, but another (Lerner, 1959) contradicted this. Sweat production by the affected skin induced by acetylcholine, pilocarpine or heat was increased (Chanco-Turner & Lerner, 1965), decreased (Halter, 1952; Ono, 1963), or normal (Gopinathan, 1965). Although these reports are confusing, segmental or zosteriform distribution of vitiligo is not uncommon and a neurological factor in the pathogenesis of vitiligo cannot be ignored. Indeed, degenerative changes in the terminal portions of peripheral nerves in the vitiliginous area have been reported using light (Yokota, 1967; Mori, 1964) and electron microscopy (Breathnach, Bor & Wyllie, 1966; Nozaki, 1976). A review of these confusing and conflicting data led to the trial of a new sweat test using physostigmine, which reflect the activity of the sweat stimulating (sympathetic) nerves more exactly than methods using acetylcholine or pilocarpine, which act directly on the sweat glands and in which the role of the sympathetic nerves would be obscured. The results of the test were variable and no conclusion could have been deduced at a glance. But when these cases were arranged taking into account the two types of clinical features (Table 1), many interesting facts were revealed. There was a significantly higher incidence of abnormal sweating in dermatomally distributed vitiligo than in non-dermatomally distributed vitiligo. Conversely normohidrotic skin was found much more often in non-dermatomally distributed vitiligo. There are 18 cases which could not be confidently placed in either the dyshidrotic or the normohydrotic group. But, with this method *in vivo* this number of unclassifiable cases is inevitable.

After the dyshidrotic cases had been divided into two types, hypohidrotic and hyperhidrotic, it was noticed that the former was more common than the latter, which was found only in cases of long duration and showing marginal hyperpigmentation. These facts show that dermatomally distributed vitiligo is associated with dysfunction of the sympathetic nerves in the affected area, decreased activity in the early stage and in some cases increased activity in the later stage. In non-dermatomally distributed vitiligo, the activity of the sympathetic nerves is unchanged. This suggests that the two types of vitiligo have differing pathogenesis. In dermatomally distributed vitiligo, the primary disturbance would occur in the sympathetic nerve and a disturbance of the melanocytes follow. After some time, the function of the sympathetic nerve might recover giving rise to marginal hyperpigmentation. The association of normal sweating with non-dermatomally distributed vitiligo suggests that the primary disturbance occurs in the melanocytes themselves. According to the author's clinical observations, the associations with halo naevi and vitiligo appearing as a Köbner phenomenon following scratches are observed exclusively in non-dermatomally distributed vitiligo. These observations suggest an autoimmune mechanism, the autonomic nervous system being not principally involved.

The two therapeutic experiments were planned to support this hypothesis. If non-dermatomally distributed vitiligo is the result of an immune reaction, corticosteroid treatment should be effective. The first person to describe the use of corticosteroid on vitiligo was Tsukada (1959), using local injections in the vitiliginous area. Farah *et al.* (1967) used oral triamcinolone in association with psoralen therapy. The author (Koga, 1970) reported the results of topical application of corticosteroid cream or ointment, as did Kandil (1970). There are some later reports of the results of corticosteroid therapy in the treatment of vitiligo and almost all of them are in agreement as to its efficacy. However, there are no reports suggesting for which types of vitiligo it is effective. The results of this study indicate that non-dermatomally distributed vitiligo responds satisfactorily to corticosteroid, but that dermatomally distributed vitiligo does not (Table 2).

The results obtained with oral nialamide are interesting. The eccrine sweat glands are innervated by cholinergic sympathetic nerves, and in the early stage of dermatomally distributed vitiligo physostigmine revealed diminution of the release of acetylcholine at the nerve endings. Does the decreased endogenous acetylcholine influence the melanocyte? In a pilot study the author used cholinergic drugs on some patients with dermatomally distributed vitiligo, without success. After extended use of oral nialamide, a monoamine oxidase inhibitor, approximately half the patients with dermatomally distributed vitiligo were partially repigmented. This result was not completely satisfactory, and therapy using nialamide alone is not recommended, but the fact that dermatomally distributed vitiligo reacts to nialamide is important when considering the pathogenesis of vitiligo. It is possible that the adrenergic sympathetic pathway plays a role in the pathogenesis of vitiligo.

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Depigmented haloes associated with the involution of flat warts

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SUMMARY

Depigmented haloes developed around involuting flat warts in two patients whose warts were treated with a 6% salicylic acid-propylene glycol gel formulation. The clinical picture was indistinguishable from that seen in halo naevus.

CASE REPORTS

Case 1. A 22 year-old white woman with multiple discrete flat warts on the upper and lower extremities and a cluster of flat warts on the left side of the forehead was given a 6% salicylic acid-propylene glycol gel formulation (Keralyt gel) to rub onto the warts every morning and at night. No change in the warts was noted for 3½ months. Then suddenly each lesion became surrounded by a ring of depigmentation (Fig. 1). The flat wart at the centre of each area of depigmentation became erythematous and prominent. Within 2 months all the warts had regressed; only circumscribed depigmented macules remained, 44 in all. The pigment returned within 4 months.

Histopathology

The central tumour is infiltrated with mononuclear cells with exocytosis of mononuclear cells into the epidermis (Fig. 2). Most striking is the severe destructive process within the epidermis, ranging from spongiosis to necrosis of groups of epidermal cells where the architecture of the epidermis is lost and only fragments of cells remain. In one such area, a microvesicle has formed. Individually hyalinized epithelial cells are seen in the spinous and granular cell layers.

The upper dermis is infiltrated by lymphocytes which are concentrated around the subpapillary blood vessels. A few larger mononuclear cells, presumably histiocytes, are seen. The upper dermis is oedematous, the papillary and subpapillary vessels are dilated and focal haemorrhage is seen in dermal papillae. A Masson silver stain on skin from a depigmented halo demonstrates the virtual absence of melanin in the epidermis.

Case 2. A 14-year-old white girl with multiple flat and common warts on the hands and flat warts on the thighs applied Keralyt gel to the lesions for 5 months. Depigmented haloes appeared around only 3 of the flat warts on her thighs and not around any of the warts on her hands. All the warts disappeared within 3 weeks after appearance of the haloes.

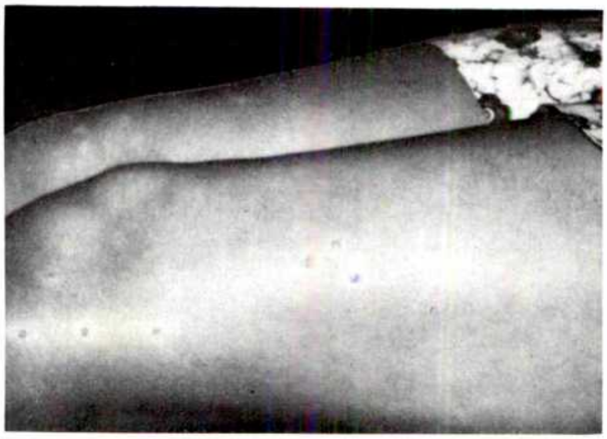

FIGURE 1. Patient 1. Depigmented haloes around flat warts.

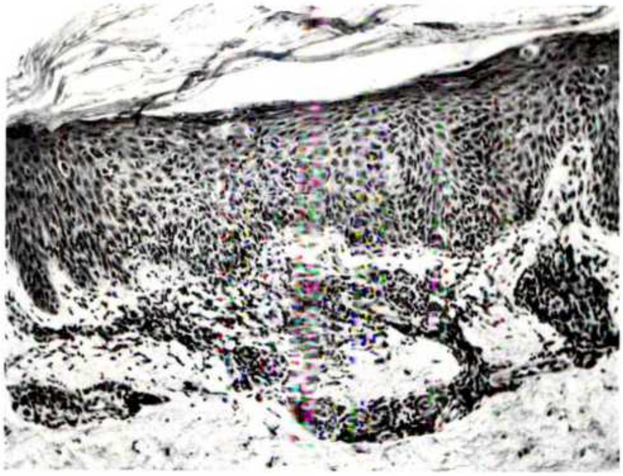

FIGURE 2. Dense perivascular mononuclear cell infiltrate in upper dermis with exocytosis of mononuclear cells and focal spongiosis and degeneration within epidermis. Patient 1 (H & E, × 160).

Studies on Keralyt gel

To determine whether Keralyt gel could produce depigmentation in normal skin, five volunteers (three white, two black) rubbed Keralyt gel into designated sites on their skin twice a day. After 4 months, only mild erythema and scaling was seen in the subjects with white skin and no change was seen in the skin of black volunteers. Occlusion of the test site with Saran wrap in one white subject did not produce depigmentation.

The two patients who developed the depigmented haloes could have had some peculiarity of their melanocytes which would predispose them to develop depigmentation after prolonged use of Keralyt gel. Both young women applied the gel to specifically designated sites on their skin twice a day. These test sites were examined at monthly intervals. After 6 months in Patient 1 and four months in Patient 2 only mild erythema and scaling but no loss of pigmentation were seen.

DISCUSSION

The designation 'depigmented halo phenomenon' is suggested to encompass all cutaneous tumours that develop haloes of depigmentation and subsequently disappear. The term 'halo nevus' should be limited to tumours which on microscopic examination are proven to be composed of naevus cells.

The histopathology in the central tumour in halo naevus is characterized by an intense infiltration of mononuclear cells intermingled with and appearing to invade the naevus cell nests and the lower portion of the epidermis (Sutton, 1916; Wayte & Helwig, 1968). Involuting flat warts show a dense perivascular mononuclear cell infiltrate in the upper dermis with exocytosis of mononuclear cells into the epidermis and focal degeneration and necrosis within the epidermis (Tagami *et al.*, 1974). The process in both appears to be similar, with the target cell being the naevus cell in halo naevi and the virus-infected epithelial cell in involuting flat warts.

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Eruptive naevi: report of two cases, with enzyme histochemical, light and electron microscopical findings

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SUMMARY

The cases of two young adults with widespread multiple eruptive naevi are described. In the first patient, more than 100 lesions appeared during a period of 6 months. Young lesions were small flesh-coloured telangiectatic papules; older lesions were larger and verrucose with histological features of cellular naevi.

In the second patient, a great number of lentigines developed in the course of 2 years. Light and electron microscopy showed that giant melanosomes were present within the lesions. The plasma level of β-MSH-like immuno-reactivity was normal.

Cases of eruptive naevi are probably not very rare, so it is perhaps surprising that only a few descriptions of the condition have been published in the English literature. Possibly the first report was that of a young woman with 'an outbreak of pigment moles' who was a patient of Jonathan Hutchinson more than a century ago (Hutchinson, 1868). Recently, Coskey (1975) described a child and two adults who presented with large crops of eruptive naevi for no known reason.

We report here the cases of two young adults who developed large numbers of naevi during a short period. Results of light and electron microscopy and enzyme histochemical studies are given in both cases. Estimation of plasma β-MSH-like immuno-reactivity has also been carried out in one patient.

PATIENTS

Case 1‡

R.E., Caucasoid man aged 20 years, who worked as a plumber, complained of a widespread eruption developing over a period of 6 months. Crops of small lesions, which were not itchy, suddenly appeared

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‡ Presented at a meeting of the Section of Dermatology, Royal Society of Medicine, 19 October 1972.

on the legs. Then large numbers of similar lesions began to develop on many parts of the body. His general health was excellent, and there was no relevant family history or history of previous diseases.

On examination, there was a widespread and symmetrical eruption mainly affecting the buttocks (Fig. 1) and backs of the upper thighs. The arms, backs of the hands and feet, lower legs and trunk were also involved to a lesser degree. The palms, soles, face and oral mucosa were spared. Early lesions resembled tiny lichenoid papules 1–2 mm in diameter; older lesions were larger, up to 1 cm in diameter, with a warty surface. Close inspection showed that many of the smaller lesions were telangiectatic. The colour of most lesions was the same as the surrounding normal skin. However, some of the larger mature lesions had become slightly pigmented. No other physical abnormality was detected. One year after the patient's first visit to the clinic, new lesions were continuing to develop. He was not seen later owing to his defaulting from follow-up.

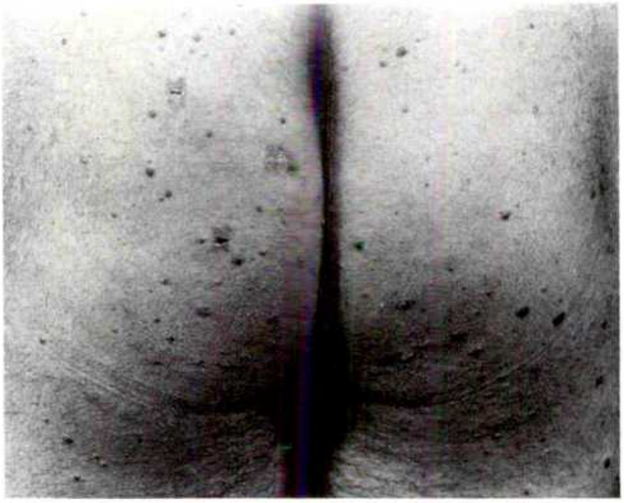

FIGURE 1. Case 1. Multiple lesions are present on the buttocks and upper thighs.

*Case 2**

A.I., Caucasoid woman, aged 24 years, gave a history of the occurrence of a large number of small brown spots all over her body during the previous 2 years. Most of the lesions had appeared in crops during the initial 9 months of the 2 year period. The patient's general health was good except for a tendency to bruise easily. Her menses started at the age of 14 years and had since been normal. She had not been receiving any drugs before the eruption first appeared. Two years prior to her first attendance at the clinic she had an operation for a receding chin. There was no family history of any dermatological, cardiac or nervous disorder.

On examination, the patient's physical and mental development were normal. Her height was 153 cm and span 166 cm. A widespread eruption of several hundred small pigmented lesions was present in both sun-exposed and covered areas, including the chest, abdomen, back (Fig. 2), buttocks, arms, neck and sides of the face. The lesions also occurred in the axillae and groins. The eruption was more prominent with a greater density of lesions on the upper part of the body (chest and arms). The lesions were mostly macular; some were slightly raised and a few had a scaly surface. The diameter of individual lesions was between 0·5 and 3·0 mm and the colour ranged from light tan to dark brown or black. Three small café-au-lait macules with irregular borders were present on the legs, and a similar lesion was situated on the neck. Examination of the cardiac, alimentary and central nervous systems showed nothing abnormal.

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* Presented at a meeting of the Section of Dermatology, Royal Society of Medicine, 17 June, 1976.

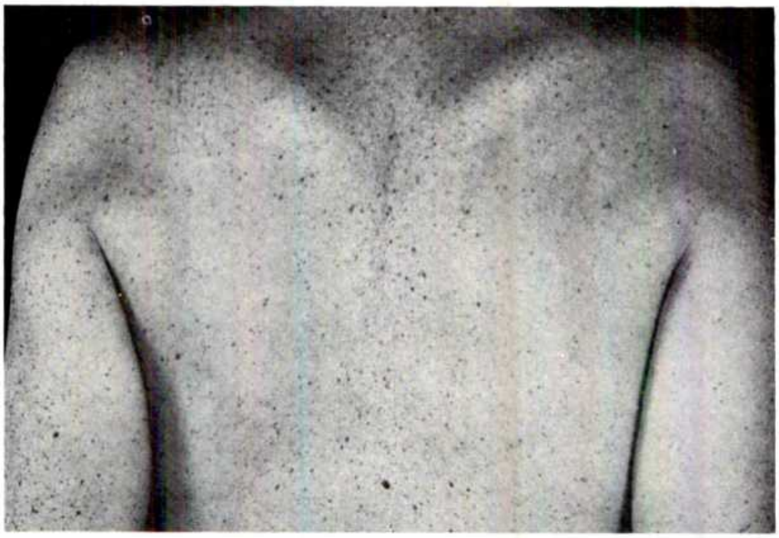

FIGURE 2. Case 2. Numerous lentigines can be seen on the arms and upper trunk.

The following investigations gave normal results: Chest X-ray; E.C.G.; haemoglobin; white blood cell count (total and differential); platelet count; euglobulin lysis time, clotting and bleeding times.

METHODS

Light and electron microscopy

Using 1% lignocaine as a local anaesthetic, excision biopsy specimens of several lesions on the buttocks were obtained in both cases. In Case 2, clinically normal skin was also biopsied for control purposes. Material from each case was processed in the following manner:

(a) For routine histology, the specimens were fixed in formol-saline and embedded in paraffin. Sections were stained with H & E and with silver nitrate (for melanin).

(b) For enzyme histochemistry, material from three lesions in Case 1 and one lesion in Case 2 was rapidly frozen in liquid N_2 or chilled hexane and sectioned in a cryostat. Reactions for the following enzymes were carried out: Dopa oxidase (Bancroft, 1967); alkaline phosphatase (Burnstone, 1961); acid phosphatase (Barka, 1960); non-specific esterase (Holt, 1954); β-glucuronidase (Hayashi, Nakazima & Fishman, 1964); ATPase (Wachstein, Meisel & Niedzwiedz, 1960); and acetyl cholinesterase (el-Badawi & Schenk, 1967). In addition, material from Case 1 was subjected to reactions for succinic dehydrogenase, NADH diaphorase and glucose-6-phosphate dehydrogenase (Pearse, 1968).

(c) For electron microscopy, the material was fixed in ice-cold collidine or veronal-buffered OsO_4 for 2 h, dehydrated in graded concentrations of ethanol followed by propylene oxide, and embedded in Epon 812. A few specimens were first fixed in 4% glutaraldehyde or half-strength Karnovsky's fixative (Karnovsky, 1965) in cacodylate buffer for 4 h at room temperature, before being osmicated.

Thin sections were collected on copper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in an AEI EM6B electron microscope.

Plasma β-MSH-like immuno-reactivity

In Case 2, the plasma level of immuno-reactive 'β-MSH' was estimated according to the method of Gilkes *et al.* (1975).

RESULTS

In Case 1, the histological features of all four lesions examined were mainly those of intradermal cellular naevi, although occasional junctional activity was apparent in some sections. In a young lesion (Fig. 3), marked dilatation of thin-walled vessels was evident in the subepidermal zone. A consistent and striking finding was the presence in the superficial and middle dermis of nests of large epithelioid naevus cells (Fig. 4 a and b) several of which possessed multiple (usually two or three) lobulated or bizarre nuclei. This abnormal appearance, coupled with the presence of occasional mitotic figures, seemed more in keeping with the histology of malignant melanoma, although the overall impression was clearly that of a benign rather than a malignant process.

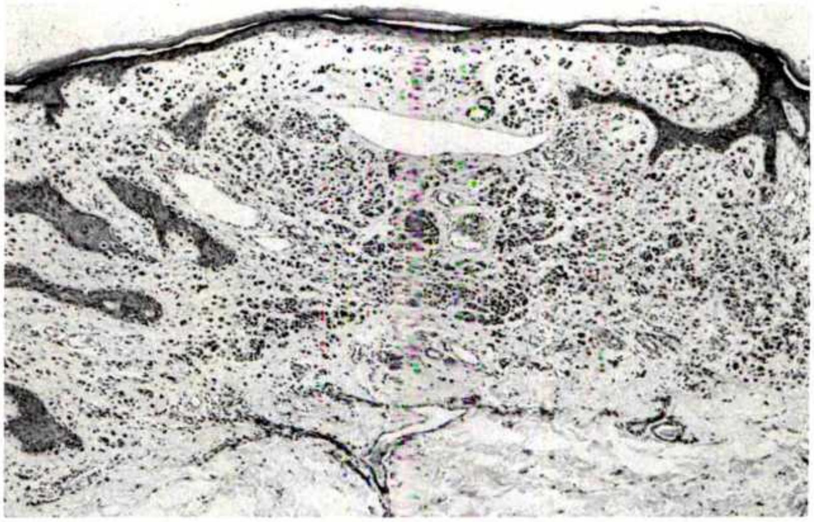

FIGURE 3. Case 1. Section of young lesion showing features of an intradermal cellular naevus with greatly dilated vessels (H & E, × 60).

The results of the enzyme histochemical reactions are summarized in Table 1. A moderate dopa oxidase activity was present in the large epithelioid cells, the reaction being strongest in the junctional or most superficial intradermal theques, and generally more pronounced than that shown by the epidermal melanocytes (Fig. 5a). The acetyl cholinesterase and alkaline phosphatase reactions were negative in the naevus cells, but some of the dilated blood vessels showed strong staining for alkaline phosphatase (Fig. 5b). The other enzymes (see Table 1) showed moderate or strong activities within the epithelioid cells.

In Case 2, the histology of all specimens was that of a simple lentigo (Fig. 6). There was some patchy thinning of the epidermis, elongation and branching of the rete processes, with an increased amount of melanin and number of basal clear cells (melanocytes) within the epidermis. Occasional nests of naevus

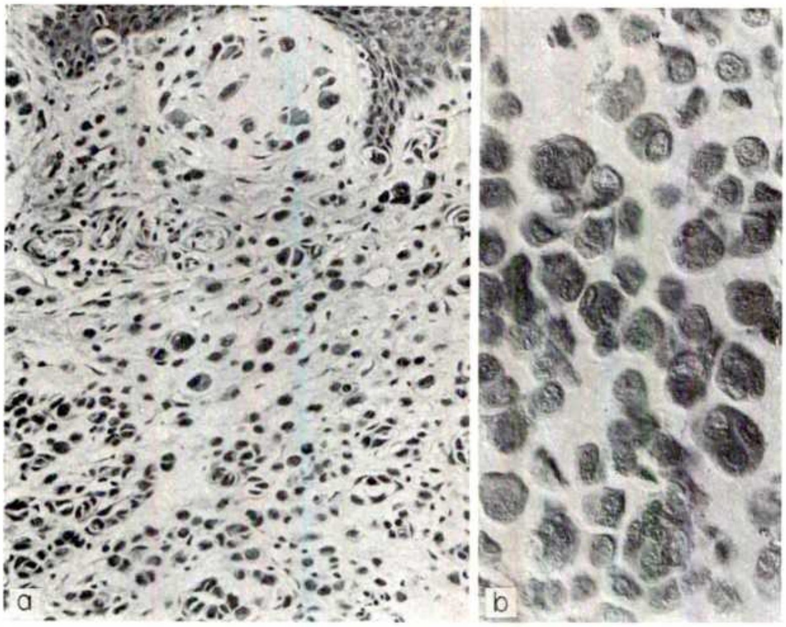

FIGURE 4. Case 1. (a) Nests of large epithelioid naevus cells are present in the superficial and middle dermis (H & E, × 150). (b) Multiple and bizarre nuclei within epithelioid cells (H & E, Normarski optics, × 380).

cells occurred at the tips of the elongated rete processes. Large globular pigment granules (giant melanosomes), appearing tan-coloured in H & E sections, were observed within the cytoplasm of the lesional melanocytes and to a lesser degree in the keratinocytes too. The largest of these pigment granules appeared in some cells to be about one quarter of the nuclear size. They were not widespread in any given section and did not occur in sections of clinically normal skin. With special stains (silver for melanin; dopa oxidase reaction) they appeared black and thus were readily identified (Fig. 7a and

TABLE 1. Enzyme histochemical findings in three lesions in Case 1

Enzyme	Activity in epithelioid cells	Other features
Dopa oxidase	+ +	Epidermal melanocytes +
Alkaline phosphatase	–	Capillary loops + +
Acid phosphatase	+ +	
Non-specific esterase	+ +	
β-Glucuronidase	+ +	Highest activity present in junctional cells
ATPase	+	Patchy activity only
Succinic dehydrogenase	+ + +	
NADH diaphorase	+ + +	
Glucose-6-phosphate dehydrogenase	+ + +	
Acetyl cholinesterase	–	Negative throughout lesions

– Negative activity; + weak; + + moderate; + + + strong.

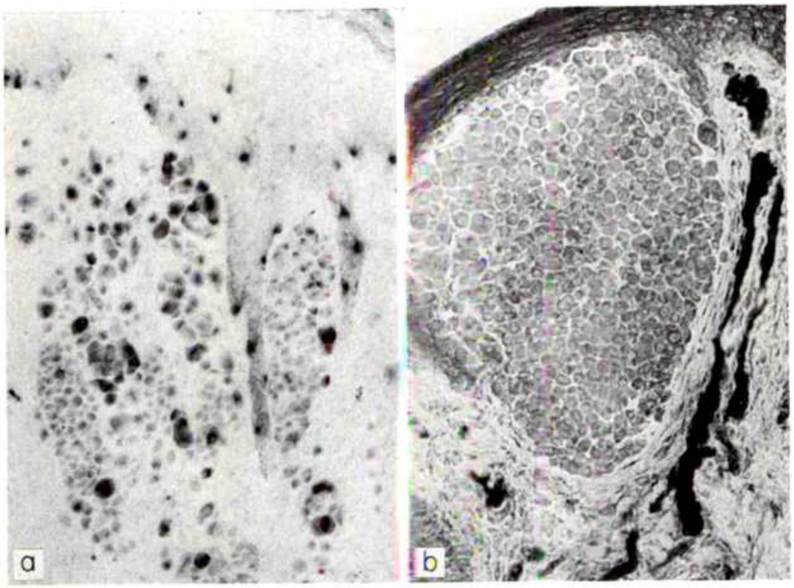

FIGURE 5. Case 1. (a) Dopa oxidase reaction. The reaction product occurs in naevus cells and normal epidermal melanocytes (× 100). (b) Alkaline phosphatase reaction. Notice localization of the enzyme within prominent blood vessel (× 120).

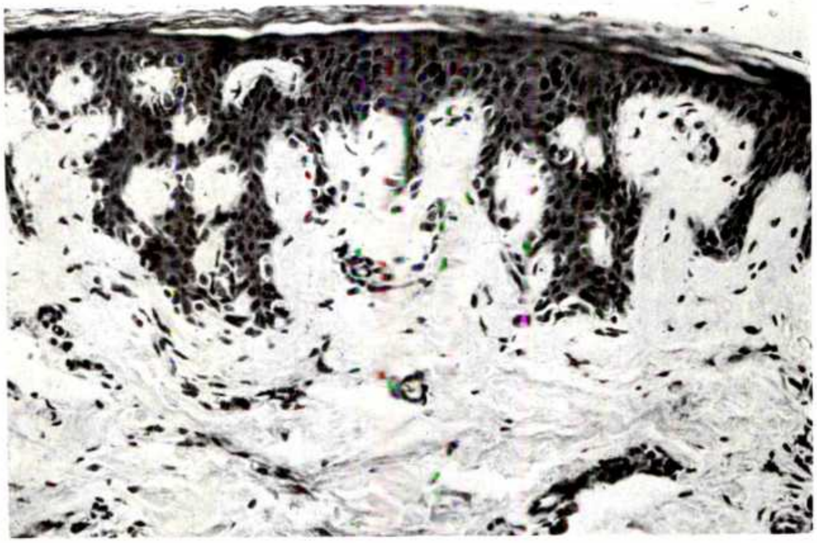

FIGURE 6. Case 2. Histology of typical lesion showing features of a lentigo simplex (H & E, × 100).

b). Apart from the demonstration with the dopa oxidase reaction of the presence of giant melanosomes and an increased number of dopa-positive melanocytes within the lesions, no essential enzyme histochemical differences were seen between the lesional and normal skin.

Naevus cell nests were not present in the dermis; no dermal abnormality was seen except for the presence of a slight perivascular infiltrate of mononuclear cells.

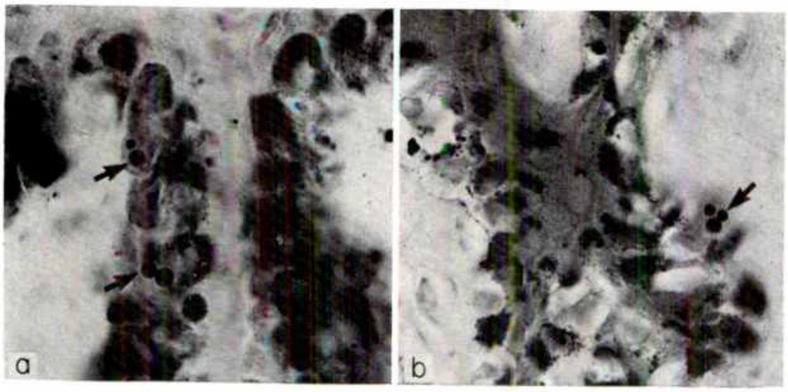

FIGURE 7. Case 2. Giant melanosomes (indicated by arrows) in histological sections. (a) Dopa oxidase reaction (× 320). (b) Silver stain (× 320).

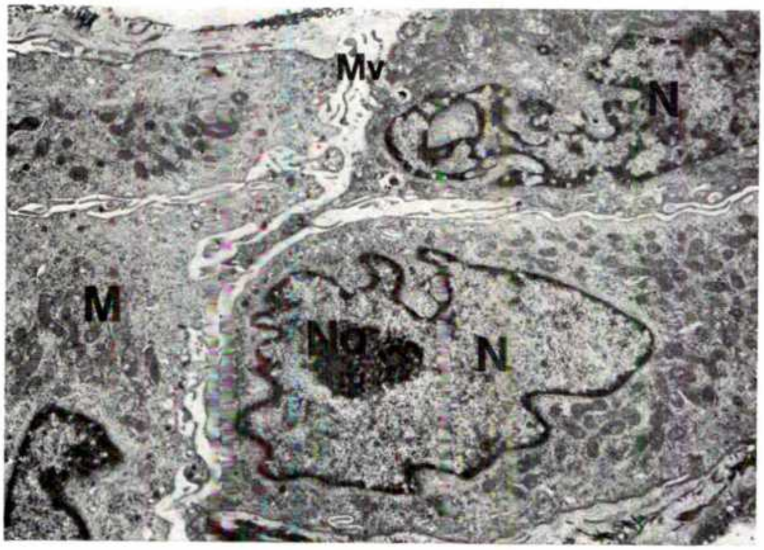

FIGURE 8. Case 1. Electron micrograph showing parts of four adjacent epithelioid naevus cells in superficial dermis. Note large indented nuclei (N) and prominent nucleolus (No.). Numerous mitochondria (M) and other common organelles are present in the cytoplasm of these cells. MV, surface microvilli (× 7000).

Ultrastructural findings

In Case 1, special attention was paid to the large epithelioid naevus cells which occurred in cell nests in the superficial dermis (Fig. 8). The nuclei of these naevus cells tended to be large and deeply indented with prominent nucleoli. The cells were usually surrounded by a basal lamina, and micro-villous processes were present along the cell surface. Most cells possessed abundant cytoplasmic organelles including mitochondria, granular endoplasmic reticulum, Golgi vesicles and polyribosomes. No abnormal organelles were seen. Heavily melanized melanosomes were also present within the cytoplasm of some of these cells and were dispersed as single units, spheroidal in shape and about 0·3 μm in diameter.

In Case 2, electron microscopy confirmed that increased numbers of epidermal melanocytes were present in the lesions. A few of these cells resembled naevus cells as they were rounded with absent dendrites. However, the majority appeared like normal melanocytes with numerous dendritic processes, parts of which were observed in the suprabasal and middle regions of the epidermis. Several of the epidermal melanocytes protruded into the dermis, and in one lesion two melanocytes were actually situated within the dermis with no evident connection with the overlying epidermis. These intradermal cells were surrounded by basal lamina.

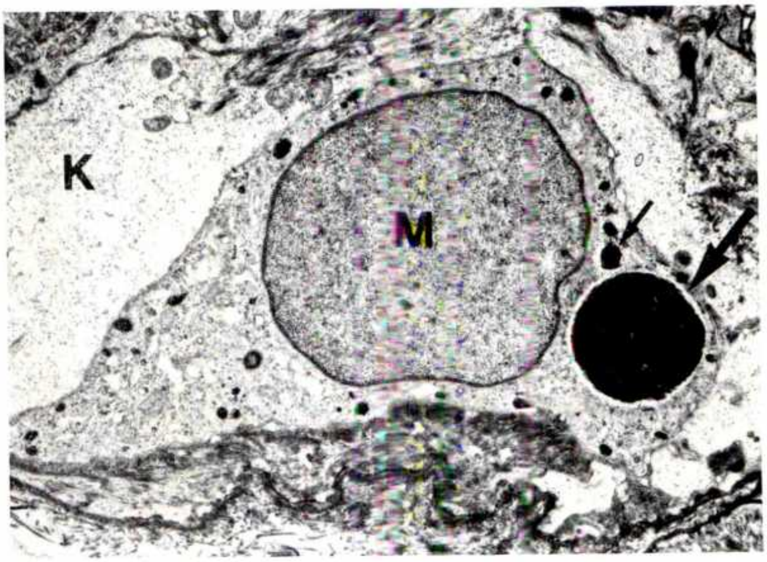

FIGURE 9. Case 2. A heavily melanized giant melanosome (large arrow) is present within the cytoplasm of a lesional melanocyte (M). This cell possesses numerous normal-size melanosomes and one of intermediate size (small arrow). The cytoplasm of an adjacent keratinocyte (K) appears oedematous in the zone nearest to the melanocyte (× 10,500).

Three distinct types of melanosome were recognized. Most frequent were normal-size ellipsoidal melanosomes, about 0·3 × 0·14 μm in size and present in abundance in both melanocytes and keratinocytes (Figs 9 and 10). Generally, these melanosomes were heavily melanized so that their internal structure was obscured by electron-dense melanin deposition. However, in a few instances (Fig. 10) the characteristic cross-striated filamentous matrix could be observed. Secondly, feather-like melanosomes, longer and thinner than the ellipsoidal variety, were also present, but in smaller numbers. Finally, a substantial proportion of melanosomes appeared to be composed of compact aggregates of vesicular structures (Figs 9 and 11b) which varied considerably in size and shape. The smallest forms were about the same size as ordinary ellipsoidal melanosomes and a few were large enough to be classed as giant melanosomes (Fig. 9), which were spheroidal or ellipsoidal in shape and consisted of a densely melanized core surrounded by a single membrane. In one instance (Fig. 11a), the internal structure of a giant melanosome was not totally obscured and concentric layers or shells of finely

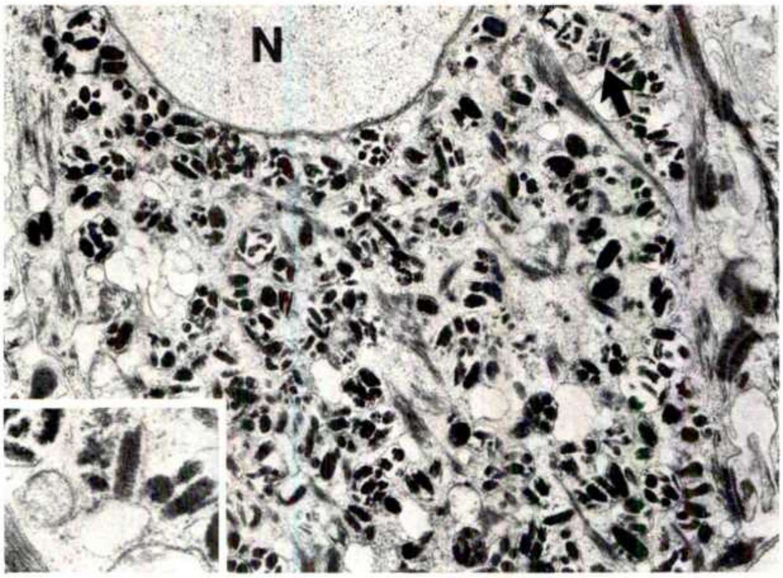

FIGURE 10. Case 2. Cytoplasm of a suprabasal keratinocyte is packed with membrane-bound complexes of melanosomes, mostly of the ellipsoidal type. N, nucleus (× 14,000). Arrow indicates cross-striated filamentous substructure of ellipsoidal melanosome (also shown in *inset* (× 38,500)).

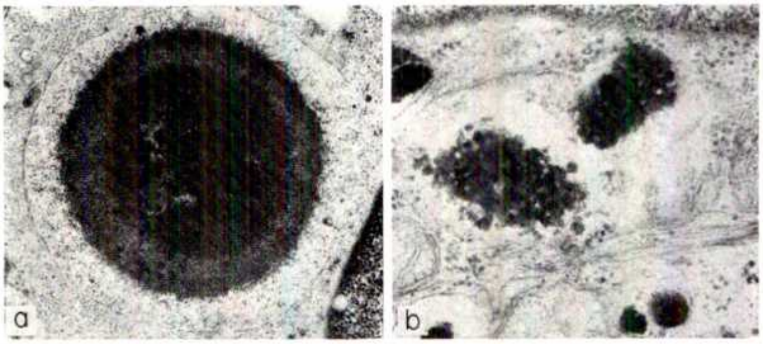

FIGURE 11. Case 2. (a) Giant melanosome in melanocyte. Concentric layers or shells of granular substructure are evident (× 19,500). (b) Two adjacent vesicular melanosomes are present in the cytoplasm of a keratinocyte (× 42,000).

grainy material were evident. The ellipsoidal and feather-like melanosomes tended to be packaged in membrane-bound complexes within the cytoplasm of the keratinocytes, whereas the vesicular melanosomes, especially the large forms, were dispersed individually. It was quite common to find all three types of melanosomes closely associated within the same melanocyte dendritic process or adjacent keratinocyte. Large membrane-enclosed aggregates of ellipsoidal melanosomes occurred in melanocytes, keratinocytes, macrophages and nerves. No specific abnormality was detected in the keratinocytes of the lesions, except the cytoplasm of the keratinocytes immediately adjacent to the melanocytes frequently appeared clear and devoid of organelles and inclusions, presumably reflecting intracellular oedema (Fig. 9).

Plasma β-MSH-like immuno-reactivity

The value was 22 ng/l and within the normal range (<15–55 ng/l).

DISCUSSION

There are notable similarities as well as differences between the two patients reported here. Both are young adults with excellent general health in whom large numbers of lesions appeared over a short period without any evident triggering factor. In both cases, however, the eruption was the expression of extraordinary but distinctly dissimilar behaviour of melanocytes or naevus cells.

In the first patient, the evolution of the typical lesion could be followed. The youngest lesions were tiny telangiectatic flesh-coloured papules which quite rapidly became larger, warty and slightly pigmented. The histological features of all lesions examined, young or mature, were those of compound or intradermal cellular naevi with nests of remarkably large epithelioid naevus cells. Enzyme-histochemical and ultrastructural analysis of these cells showed signs of heightened metabolic activity. The large epithelioid naevus cells showed marked respiratory and hydrolytic enzyme activities, certainly more prominent than we would expect to find in ordinary compound or intradermal cellular naevi (Wilson Jones & Eady, unpublished observations). In an extensive analysis of naevus cell naevi, Petzoldt (1967) noted that the activities of enzymes involved in glycosis, the citric acid cycle and respiratory chain were highest in junctional naevus cells as compared to intradermal or more deeply situated cells. Paul & Illig (1974) confirmed the observation that the activities of certain enzymes appeared to diminish in deeper naevus cells and it is of particular interest that these authors noted that peculiar 'giant' naevus cells, occurring in only some of their specimens, possessed especially marked activities of various dehydrogenases. It may be that these 'giant' cells correspond to the very large epithelioid naevus cells present in our material. We are unaware, however, of any previous account of enzyme studies on eruptive naevi as shown in our Case 1.

The fine-structural appearance of the naevus cells is somewhat similar to that described previously (Gottlieb, Brown & Winkelmann, 1965), except in our material the rough endoplasmic material was more developed, probably indicating that the cells were highly stimulated.

Sanderson (1960) described the case of a young man in whom an eruption of cellular naevi abruptly developed after a transient loss of consciousness, which may or may not have been related to the aetiology of the rash. The remarkable feature of his case was that the lesions first appeared as telangiectatic macules or papules which gradually became more raised and darker. Certainly, the evolution of the lesions in Sanderson's patient is rather similar to the course followed by the naevi in our Case 1. In his case, however, the lesions did not occur on the hands and feet as in our patient.

Multiple juvenile melanomata may also present in an eruptive manner. In one patient who has been followed for many years (Wallace, 1974) the morphology of the lesions varied considerably. In the early stage of the disease, the histology of the individual lesions showed features of malignant melanoma; later the appearance was more like benign juvenile melanoma. In another case (Capetanakis, 1975), the lesions were multiple but the mode of onset of the eruption was not reported. It is noteworthy that both patients presented as fit young adults with the eruption affecting the buttocks, as demonstrated in our case. However, 6 years after the onset of his eruption, the patient of Capetanakis (1975) developed frequent epileptic fits without obvious cause, and the question is again raised as to whether the neurological and cutaneous features can be related.

In our second case, the lesions were clearly lentigines and therefore quite unlike the naevi of our first case. Although occasional small nests of naevus cells occurred at the tips of the rete ridges in the lesions, in none of the sections examined were the theques large or frequent enough to designate the lesions as true junction naevi. Here, the most interesting microscopical feature was the presence, in all three lesions biopsied, of giant melanosomes (or macromelanosomes) mainly within the melanocytes but also in keratinocytes and macrophages. The fine structure of these organelles has been described in detail elsewhere (Jimbow, Szabo & Fitzpatrick, 1973; Konrad, Wolff & Honigsmann, 1974). Zeisler and Becker (1936) found 'large pigment globules' in the lesions of a patient with generalized lentigo. In a photomicrograph (Fig. 4 in their paper) these pigment masses are shown in a section of a typical lesion from their patient. They are so similar to the granules now known as giant melanosomes or macromelanosomes that we believe that this may actually be the first description of these important cellular organelles. Szabo (1959) and Benedict *et al.* (1968) first drew attention to the occurrence of the giant pigment granules in the skin in neurofibromatosis, and more recently these granules have been identified in naevus spilus (Konrad, Wolff & Honigsmann, 1974; Konrad, Honigsmann & Wolff, 1974; Eady, Sparrow & Grice, 1975), cellular naevi, and even normal skin of healthy individuals (Konrad & Honigsmann, 1975). We believe to be of special interest the observation of giant pigment granules in the lesions of two cases with lentiginosis profusa (multiple lentigines) syndrome (Selmanowitz, 1975), which provides a common sub-cellular link between the eruption affecting our patient and the serious hereditary cardio-cutaneous disorder. It should be stressed that we could find no evidence of cardiac or other internal disturbance in our patient and that the lesions were unlike those uniformally black 'ink-blot' macules typically found in the multiple lentigines syndrome.

Crops of moles may appear or existing ones may darken during puberty or pregnancy thus pointing to possible hormonal influences on pigment cell behaviour (Coskey, 1975). We were able to measure the plasma level of melanotrophic hormone ('β-MSH') in Case 2, and obtained a normal result.

ACKNOWLEDGEMENTS

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Light sensitivity in mycosis fungoides

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SUMMARY

Because of suspected photosensitivity, twelve patients suffering from mycosis fungoides have been phototested with various wavebands. Four patients experienced a burning sensation and erythema following sun-exposure, after the disease had progressed to stage II. On phototesting, abnormal reactions were observed in 7 patients. The offending wavebands were situated mostly in the UVA range, but also in the UVB range and in visible light.

Mycosis fungoides (MF) is generally regarded as a malignant lymphoma always appearing first in the skin. The disease has classically been divided into three stages (premycotic, infiltrative and tumour stages). It can affect the skin for a period varying from a few months up to 30 years before affecting the lymph nodes and internal organs. Studies of peripheral blood and lymph nodes have brought some evidence that the neoplastic cells are thymus-derived (T) lymphocytes.

The aetiology of the disease is unknown. Many patients, however, relate its appearance to a drug or an allergic reaction, and an allergic component can often be identified. Tan *et al.* (1974) supported the hypothesis that MF is a chronic granulomatous response to persistent unidentified antigen(s), upon which immune imbalance can develop resulting in 'auto-immune' phenomena, and in a few cases leading to the emergence of various lymphoreticular neoplasms.

Light sensitivity in MF has drawn very little attention, and since one MF patient with light-sensitivity appeared at our clinic about 1 year ago, we have discovered by phototesting that several cases may have pathological light-reactions. During the last year we have collected and phototested 12 MF patients. The results are compared with the data obtained in 22 normal subjects.

MATERIALS AND METHODS

Twelve patients were investigated, all having a clinical history and appearance typical for MF. The diagnosis was verified histologically in all patients. The group consisted of 5 women and 7 men aged 42–78 years, the average being 61 years. Nine of them were classified in stage II (plaques or infiltration) and three in stage III (tumours) (Table 1). All had normal routine haematological and histochemical tests. Four patients had a history of reactions to sunlight and experienced pruritus and dermatitis on exposed areas.

TABLE I. Sex, age distribution and stage of disease in 12 patients with mycosis fungoides

Case	Patient	Sex	Age	MF-stage
1*	R.O.	F	59	II
2*	E.S.	F	57	II
3	M.H.	M	68	III
4	R.P.	M	50	II
5	A.S.	F	57	II
6	E.J.	F	69	III
7*	J.H.	M	54	II
8	A.L.	M	56	II
9	O.O.	M	63	II
10*	O.A.	M	78	II
11	M.T.	F	42	III
12	F.F.	M	78	II

* Anamnestically sensitive to sunlight.

Case 1. R.O., female, aged 59 years, housewife, brunette. From 1973 she had had widespread erythematous spots on the body and extremities. From January 1975 increasing infiltrated plaques appeared, especially on the upper part of the body and on the upper limbs. The diagnosis MF stage II was confirmed both clinically and histologically in August 1975. During the summer of 1975 she noticed a slightly desquamating dermatitis on sun-exposed areas, and also slight itching and burning on the rest of the body.

Case 2. E.S., female, aged 57 years, innkeeper, blond. Previously she had been healthy. From the beginning of 1974, intermittent erythema and eczematous lesions developed over her body. From April 1975 the lesions on the body increased to infiltrated plaques the size of a child's hand. The diagnosis MF stage II was confirmed both clinically and histologically in August 1975. During the summer of 1975 she noticed burning and erythema on the face after sun-exposure. She had never sunbathed very much and had not previously experienced direct discomfort.

Case 7. J.H., male, aged 54 years, industrial worker. He had had pulmonary tuberculosis in 1950, and since then no sign of relapse. He had been an alcoholic for some years, but for the last 3 years a teetotaller. From 1960 intermittent psoriasis-like skin lesions had appeared on his body. From January 1975 increasing infiltrated plaques, up to 7–8 cm in diameter, had been present on his body and lower limbs. The diagnosis MF stage II was confirmed both clinically and histologically in June 1976. During the summer of 1976, he noticed itching and a burning feeling, accompanied by erythema and slight oedema, on sun-exposed areas after sun-bathing.

Case 10. O.A., male, aged 78 years, retired manager. Intermittently from 1963 he had noticed erythematous areas on his body, at first interpreted as seborrhoeic eczema. From 1973 abundant widespread coin-sized infiltrated plaques appeared on his body, and also a few on the extremities. He had always sunburned easily, but in the last 2–3 years he had been even more sensitive and developed erythema on sun-exposed areas accompanied by itching and burning. On examination in September 1976, he had a well-defined erythema on his face and the backs of his hands.

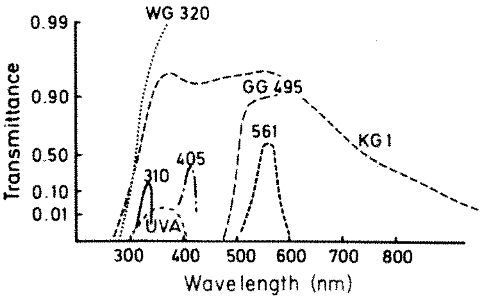

FIGURE 1. Transmittance of the individual filters as measured spectrophotometrically.

Twenty-two subjects, males and females, aged 17–54 years, served as controls and were tested in the same way as the patients.

Light testing procedure

Normal skin (size of radiated area = 10 mm diameter) was phototested with an Osram High Pressure Xenon Arc Lamp (XBO = 150 W) without any mirror or lens focussing system and equipped with different Schott filters. The lamp was placed at a distance of 20 cm from the patient's skin. All tests were performed on the back. Energy output was measured prior to each exposure with a Hewlett & Packard Radiant Flux Meter. Transmission characteristics were measured spectrophotometrically (Fig. 1). The following filters were used: KG$_1$+310 (interference filter), half bandwidth (h.bw.) 305–315 nm and tenth bandwidth (t.bw.) 301–317 nm; KG$_1$+WG 320, h. bw. 345–745 nm; KG$_1$+WG 320+UG 5 (i.e. UVA) h.bw. 350–390 nm, KG$_1$+405 (interference filter), h.bw. 397–412 nm and t.bw. 391–417 nm; KG$_1$+GG 495 h.bw. 495–745 nm; KG$_1$+561 (interference filter) h.bw. 535–585 nm and t.bw. 518–597 nm. Thickness of the cut-off filters was: KG$_1$, 2 mm; WG 320, 3 mm; UG5, 2 mm; GG 495, 2 mm.

The mean dose rate (or irradiance) at 20 cm from the unfiltered Xenon Arc Lamp was 210 mW.cm^{-2}. The KG$_1$ filter was used mainly to reduce heat from the strong lines in Xenon emission at 800–1000 nm.

The range of exposure times used for the different filter combinations was approximately: KG$_1$+310, 20–200 s; KG$_1$+WG 320, 30–360 s; KG$_1$+WG 320+UG5, 10–40 min; KG$_1$+405, 10–30 min; KG$_1$+GG 495, 5–15 min; KG$_1$+561, 10–30 min.

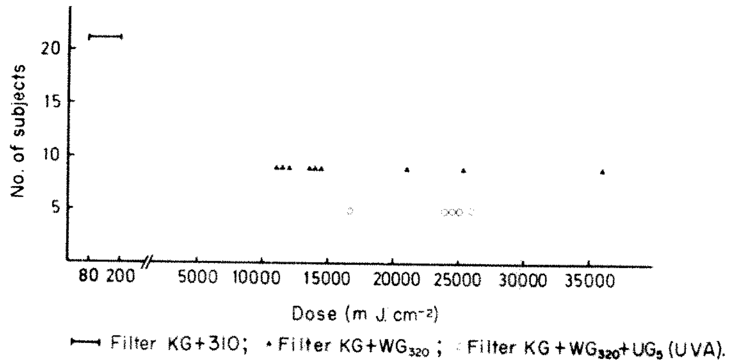

FIGURE 2. Minimal erythema dose (MED) in 22 normal subjects (doses in mJ·cm^{-2}).

The typical irradiance with the Xenon Arc Lamp read with the Hewlett–Packard Meter for each filter combination was approximately: KG_1+310, 1·2 mW.cm^{-2}; KG_1 + WG 320, 130 mW.cm^{-2}; KG_1 + WG 320 + UG5, 9 mW.cm^{-2}; KG_1+405, 0·5 mW.cm^{-2}; KG_1 + GG 495, 80 mW.cm^{-2}; KG_1+561, 5 mW.cm^{-2}.

The dose which gave a faint but distinct erythema, minimum erythemal dose (MED) and an apparent threshold papular response (PR) were recorded at 8, 24 and 48 h after irradiation.

RESULTS

Controls

Fig. 2 shows the doses corresponding to the MED obtained in 22 normal subjects. With the KG_1+310 nm interference filter combination all 22 subjects reacted with erythema after doses of 80–200 mJ.cm^{-2}. With the KG_1 + WG 320 filter and 11–36 J.cm^{-2} erythema occurred in nine normals, whereas the others did not react to 36 J.cm^{-2}. In the UVA spectrum (KG_1 + WG 320 + UG5 filters) 5 had reactions from doses of 16·8–26·0 J.cm^{-2}, while the others did not react to the highest dose tested, i.e. 26 J.cm^{-2}. With all the above mentioned filter combinations the erythema was delayed and appeared at 8 hours or later. Thus it was interpreted as probably a non-thermal response. None of the controls showed a papular response. No reactions were obtained with wavelengths above 400 nm using the different filter combinations mentioned above.

TABLE 2. Abnormal reactions (MED and PR) in patients with mycosis fungoides (doses in mJ.cm^{-2})

	Filters									
Case	KG_1+310		KG_1 + WG 320		KG_1 + WG 320 + UG 5		KG_1+405		KG_1 + GG 495	
	MED	PR	MED	PR	MED	PR	MED	PR	MED	PR
1			9000	18000	12600	DNI				
2			8400	16800	12600	DNI				
3										
4	60	120	8400	16800	13500	DNI	900	DNI	60000	DNI
5										
6										
7	60	DNI	(14000)	DNI			900	DNI	25200	DNI
8				19200	15300	DNI				
9			9000	18000		14400				
10	30	90	(15600)	DNI	(21600)	DNI				
11										
12										

DNI = Dose not increased.

The figures in parentheses are considered normal, but are included to show the relation with the other wavelengths to which the patients reacted abnormally. Cases 3, 5, 6, 11 and 12 showed no abnormal reactions.

Patients

Table 2 shows the MED and the PR considered to be pathological in MF patients in relation to the normal controls. Three patients had pathological reactions with the KG_1+310 filter: 30–60 mJ.cm^{-2}; five patients reacted pathologically with the KG_1 + WG filter: 8·4–9·0 (19·2 PR) J.cm^{-2}; five patients had pathological reactions with $KG_1+WG+UG5$ filter: 12·6–15·3 J.cm^{-2}; while two patients had erythema to visible light and this can be considered an abnormal reaction (Cases 4 and 7).

When the test dose was increased by ca. 100% above the MED (Table 2) in five patients, they all showed a papular reaction, in addition to the one who reacted with a papular reaction to the original dose. In total 7 of 12 MF patients had pathological light sensitivity and in 6 of the 7, PR occurred when the test dose was doubled. The 7th patient developed the PR without increased dosage.

Two patients (Nos 4 and 10) showed a papular reaction with irradiation through the KG_1+310 nm interference filter combination to doses of 90–120 mJ.cm^{-2}. Such a response was never seen in the controls. The MEDs of 3 patients (nos 4, 7 and 10) were from 30–60 mJ.cm^{-2} which dose range was below that found in normals.

With the $KG_1+WG\,320$ filters 5 patients (nos 1, 2, 4, 8 and 9) showed a papular response at 16·8–19·2 J.cm^{-2}. Again this response was not seen in the control subjects. It may be noted that one patient (No. 8) reacted with a papular response but not with erythema alone. But 4 others reacted with erythema to smaller doses than that causing a papular response and had MEDs of 8·4–9·0 J.cm^{-2}. This MED range is again just below that found in the normals.

With the filter combination giving UVA, 4 patients (nos 1, 2, 4 and 8) had MEDs of 12·6–15·3 J.cm^{-2}, which were also just below the values found in those normals that reacted to this part of the spectrum. Another patient (No. 9) showed a papular response to UVA.

Two patients (Nos 4 and 7) reacted to visible light with erythema, whereas none of the controls did so. Although we were not able to test all patients to all filter combinations and give doses above the observed MEDs, it may be noted that 6 patients developed papular responses on irradiation at one part or another of the spectrum, in most instances with doses 2–3 times above their MED. Thus, 7 of 12 MF patients had some evidence suggestive of abnormal photosensitivity, in 6 because of a pathological morphological response, in 1 (No. 7) because of the development of erythema to visible light with doses that were without action in normals.

In conclusion we noticed that approximately 40% of the MF patients had an action spectrum involving UVA and in a minor degree UVB and visible light. The small number of patients must, however, be taken into account.

DISCUSSION

The investigation of patients with light sensitivity is intended to determine whether they respond to light of an appropriate wavelength in a manner which differs from normal. From the small group presented here, it is likely that many patients with MF suffer from photosensitivity over a wide wavelength range. Light sensitivity in MF has previously drawn very little attention. Ive (1964) reported one case, and Van Scott & Yu (1975) described another case with associated photosensitivity in which phototests confirmed his markedly low threshold erythema dose for both UVA and UVC in normal skin. Kobori *et al.* (1974) described 5 actinic mycosis fungoides cases, and by using monochromatic irradiation tests found that an erythematous reaction was easily provoked in the UV-area 310–350 nm. It seems very likely that these patients represented actinic reticuloid rather than MF.

Several authors have described photosensitivity in diseases related to MF. Wiskemann (1965) reported a case of light provokable granulomatous reticulosis. Jensen & Sneddon (1970) described a case of actinic reticuloid which was associated with reticulum cell sarcoma. It has been recognized for many years that patients with cutaneous lymphocytomata may show evidence of associated photosensitivity. Frain-Bell & Magnus (1971) reported two cases of cutaneous lymphocytomata associated with solar urticaria and polymorphic light eruption. Phototesting results suggested that the action spectrum in each case was probably dependent on the type of associated photodermatosis and not on the lymphocytoma. Lutzner (1975) has indicated that Sézary's syndrome, which is closely related to

MF, most often begins on the face, and that the skin sometimes develops infiltrated lesions. These usually occur on the face, but do not mean that light is necessarily associated with the disease, even if it may seem suspicious.

The four MF patients with light sensitivity stated that they reacted abnormally to light only after being classified in stage II. None of these patients appeared to have been exposed to photoactive substances, or to have suffered from systemic disease, other than MF, or its treatment. There was no clear history of photodermatosis in these 12 patients, except in one case. This patient described sharply defined erythema on sun-exposed areas, while 3 others developed only faint and less definable erythema and a burning sensation on sun-exposed areas. Nevertheless, there had been a clear provocation by sunlight. The last 3 patients could hardly define the time relationship between sun-exposure and the subsequent skin reaction. All 4 patients got worse during the most sunny months, but they did not notice anything abnormal in the winter.

By systematic phototesting of these MF patients, independent of a history of light sensitivity, we discovered in about one half of them a decreased MED over a wide action spectrum involving UVB, UVA and visible wavelengths, with most pathological reactions in the longwave UV-area. Even if the MED was just below normal, clearly pathological reactions such as PR were obtained in a high percentage at all UV wavelengths when the exposure was increased to double the MED.

We do not know the reason for light sensitivity in MF, but it was striking that the patients did not develop abnormal responses until the disease had reached stage II. Knowing that it is not possible histopathologically to find abnormal cells before stage II, one might think these cells to be entirely or partly responsible for the light sensitivity in MF. Presumably these reactions involve some mediator system which also functions abnormally in apparently normal skin, since all tests were performed on unaffected areas.

Even without coming closer to the pathogenesis, we think that MF and actinic reticuloid (AR) have many striking features in common clinically, histologically and photobiologically. Both diseases start in a similar manner as a non-specific dermatitis, even if the light factor is considerably more apparent in AR than in MF. The diseases differ in that AR does not develop lymphomas even if the histological picture of the skin may resemble a lymphoma with abnormal cells from the lymphocyte series. Also in AR the patients react more strongly to longer wavelengths, such as visible light.

Even if the photobiological results of this study are by no means conclusive, they indicate a relationship between mycosis fungoides and light sensitivity.

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Influence of wind on chronic ultraviolet light-induced carcinogenesis

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SUMMARY

Wind enhances the carcinogenic effect of chronic ultraviolet radiation (UVL). This was demonstrated in hairless mice that were irradiated for 42 weeks with mercury arc lamps. One group of animals was exposed to continuous wind flow of 2·7 m/s except for the daily 1–2 min time interval when they were removed from the wind tunnel and irradiated. Another group of animals received identical irradiation but were protected from wind. The first tumour appeared in the UVL and wind group after 105 days of irradiation, and at 164 days of irradiation all surviving mice in the group had developed tumours. The group of mice receiving identical irradiation but protected from wind had their first tumour appear at 154 days of irradiation, and by 164 days of irradiation only 40% of the mice had developed tumours.

Considerable effort has been devoted to the study of ultraviolet light (UVL) injury (Blum, 1959; Urbach, 1969) but with the exception of the study on the effect of heat (Freeman & Knox, 1964), the role of environmental parameters was largely ignored until this laboratory reported on the influence of wind (Owens *et al.*, 1974) and humidity (Owens *et al.*, 1975) on acute ultraviolet injury. This report relates to the influence of wind on UVL induced carcinogenesis.

MATERIALS AND METHODS

Young adult female hairless mice weighing approximately 25 g were maintained in an environmental chamber under the conditions of 20% relative humidity at 32°C. All mice were housed in stainless steel cages and had free access to commercial mouse chow and tap water. Three mice were in each cage and all groups of mice were exposed to the same fluorescent room lighting. One group of mice (30 animals) received irradiation 5 days weekly from 2 mercury-arc lamps.* The animals were removed

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*GE Uviarc Model US-3 available from General Electric Co., Cleveland. The lamps have a 360 watt rating, are housed in a quartz tube, and have a broad spectrum emission (220–280 nm, 10·36 W; 280–320 nm, 13·72 W; 320–400 nm, 12·23 W; 400–760 nm, 25·10 W).

from the chamber for irradiation and immediately returned after receiving the UVL. A second group (30 animals) received irradiation in an identical fashion to the first group but, additionally, were maintained in a wind tunnel within the chamber and exposed to constant wind flow of 2·7 m/s. Wind velocity in the tunnels was measured with a vane instrument as reported in a previous publication (Owens *et al.*, 1974). A third group of mice (6 animals) maintained in the chamber were exposed to constant wind flow of 2·7 m/s but not irradiated.

The irradiated animals received a sub-erythemal dose of UVL daily 5 days weekly for 42 weeks. The animals were exposed to an increasing ultraviolet dose in order to accommodate thickening of the skin with continued exposure. The intensity of the lights was measured, using an Eppley Thermopile calibrated against a National Bureau of Standards standard lamp, to be 723 mW/cm^2 at the site occupied by the animals. On the first day the animals were given a dose of 37·6 mJ/cm^2 (52 s) and on day two a dose of 21·7 mJ/cm^2 (30 s). This was enough to cause reddening of the skin so that no radiation was given for the next 4 days. Thereafter, a daily dose of 21·7 mJ/cm^2 was given for 18 irradiation days (i.e. 5 days per week with no radiation at weekends); then increased to a daily dose of 28·9 mJ/cm^2 for 20 irradiation days; then increased to a daily dose of 43·4 mJ/cm^2 for 50 days; and finally increased to a daily dose of 54·2 mJ/cm^2 for 70 days. The experiment was terminated after a total of 164 irradiation days. The first tumour appeared in the group exposed to UVL and wind at a total cumulative dose of 3·80 J/cm^2. The first tumour appeared in the group exposed to UVL but no wind at a total cumulative dose of 6·45 J/cm^2. After the reddening that occurred on day 2 and faded by day 6, no further erythema was observed. The animals were examined weekly for development of skin lesions. The development of a discrete tumour 2 mm in diameter was considered the end point. Total numbers of tumours or numbers of tumours per animal were not recorded. The tumours were excised for histopathological study in a random fashion in 20% of the animals.

RESULTS

The first tumour appeared in the group tested with UVL and wind at 105 days of irradiation, and by 164 days, all surviving mice in the UVL and wind group had developed at least 1 tumour (Fig. 1). In contrast, the group exposed to UVL but not wind had the first tumour appear at 154 days of irradiation and by 164 days only 40% of the mice had developed tumours. The difference in the two

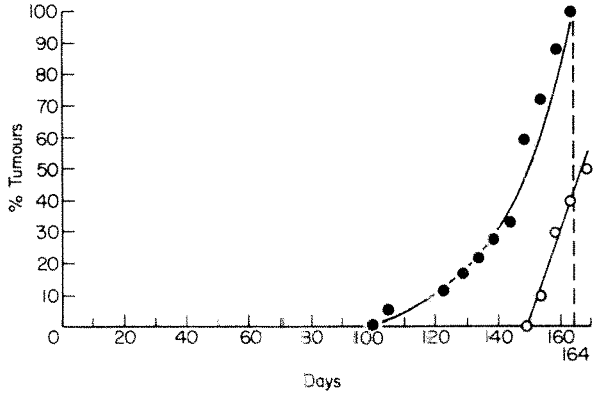

FIGURE 1. Prevalence (mice with tumours/number of surviving mice). (●) Wind plus UVL; (○) UVL only.

groups was statistically significant from day 124 ($P<0{\cdot}05$) (chi-square test). As the experiment progressed and more tumours developed, the significance increased to $P<0{\cdot}001$ by day 164.

All the tumours examined histologically were squamous cell carcinomas. None of the animals exposed to wind alone developed tumours.

Survival of animals in the irradiated group is shown in Fig. 2. None of the non-irradiated animals was dead at the conclusion of the experiment. Unfortunately, 12 animals died in the irradiated, wind exposed group after 76 days of irradiation. This resulted from a failure of the thermostat in the chamber with cooling over one weekend. We have observed this previously in animals cooled and exposed to wind. Since cooling of animals protects them from the effects of UVL, we do not believe the isolated event accelerated tumour formation among the surviving animals.

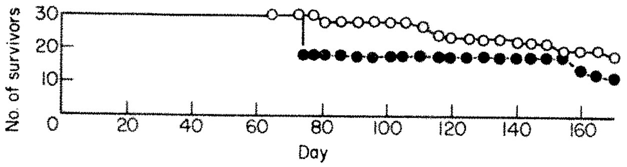

FIGURE 2. Survival of animals in each group. (●) Wind plus UVL; (○) UVL only.

DISCUSSION

It has been shown previously that heat enhances the injurious effect of UVL in both acute UVL injury and carcinogenesis (Freeman & Knox, 1964). Earlier experiments (Owens *et al.*, 1975) also demonstrated that high humidity, water immersion, and application of wet packs produced greater irradiation damage than when animals were maintained in a dry environment.

Previously we have studied the influence of wind on albino mice (Owens *et al.*, 1974) irradiated over a 4-week period. Little difference was seen between irradiated animals protected from or exposed to wind until the second week. By this time damage to the animals' ears had reached a peak, and the animals began to recover in spite of continued irradiation at the same dosage. The animals exposed to wind and UVL had slightly greater damage at the injury peak than did those exposed to UVL alone, but even more impressive was the delayed recovery in animals exposed to wind while irradiated.

Since wind adversely influences UVL induced damage over a 4-week period, it was not surprising that it also promotes tumour development. We do not believe that wind exposure led to increased skin exposure to trace chemical carcinogens in the atmosphere as the chambers were housed in an air conditioned room and the air provided to the environmental chambers is filtered room air. There are no other experiments taking place in the room where the environmental chambers are housed.

It is apparent that wind promotes UVL induced carcinogenesis; however, we are not prepared to speculate on the specific pathophysiological mechanisms responsible for this effect.

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Chronic leg ulcers treated by the pinch graft method

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SUMMARY

Forty-nine patients with chronic leg ulcers were treated by pinch grafting. The initial success of this procedure was high for both gravitational and arterio-sclerotic ulcers. Over half the grafted gravitational ulcers and 70% of grafted arteriosclerotic ulcers were intact after 2 years. Some grafts remained stable for 6 years. Arteritic ulcers did not respond as well; none of the grafted ulcers survived more than 6 months.

Pinch grafting is still a simple, cheap and effective therapy offering prolonged graft stability as well as quicker healing to those patients who are not fit for more extensive surgery.

Chronic leg ulceration is common. Gravitational ulceration alone is found in 1–1·5% of the general population (Editorial, 1975). In Leeds, in the past 5 years, we have seen an average of 127 new patients per year with chronic leg ulceration caused by either chronic venous or arterial disease. This represented approximately 2% of all new patients. Leg ulcer dressings accounted for 35% of the annual attendances at special dressing clinics (excluding psoriasis), and 10% of all visits to follow-up clinics. Further, leg ulcer patients occupy 28% of the in-patient beds per year.

The reasons for this major demand upon the resources of the Department are not hard to see. Although some patients are referred for vascular surgery, the majority are unfit because of chronic diseases such as obesity, ischaemic heart disease or chronic bronchitis. Those patients with arterio-sclerotic peripheral vascular disease respond poorly to medical treatments and may not be fit for the surgical therapy which would give them long term benefit. There is a small proportion of patients who are not able to cope with the treatment of their ulcers through ignorance or apathy.

Conservative therapy with topical treatment is time consuming, and on economic grounds vastly wasteful, given the cost of in-patient hospital therapy. Pinch grafting of leg ulcers is a minor surgical procedure which has been used successfully in the past for the treatment of chronic gravitational ulcers (Davies, 1930; Reiter, 1948; Chilvers & Freeman 1969; Smith, Holder & Smith, 1971). This procedure does not subject patients to the hazards of a general anaesthetic and can be performed simply on the ward with no elaborate equipment.

Pinch grafts were originally described by Reverdin (1872) who cut grafts consisting mainly of epidermis but other techniques (Davies, 1930) have used small full thickness grafts or large whole grafts which have been divided subsequently into smaller sections (Gabarro, 1943).

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We considered that the Reverdin type graft would accelerate the healing of leg ulcers and reduce the morbidity and subsequent clinic attendances of those patients with large and small gravitational or arterial ulcers.

METHOD

The leg ulcers of 49 patients were treated by the pinch graft method after the surface of the ulcers had been prepared by dressing with eusol soaks to produce a clean, granulating surface. There were 33 gravitational ulcers in 9 males age 38–87 years (mean age 62·3 years) and 22 females aged 41–86 years (mean age 65·1 years). In a group of 14 arteriosclerotic ulcers there were 13 females aged 60–87 years (mean age 71 years) and one male aged 67 years. Four female patients aged 52–75 years (mean age 59·5 years) had either Felty's syndrome (2), rheumatoid arthritis (1), or necrobiosis lipoidica (1).

The antero-lateral aspect of one thigh was used as the donor site and locally anaesthetized with 2% plain lignocaine. Pinch grafts, the diameter of which varied between 0·2 and 0·4 cm were obtained by using a Hagedorn needle, Kilner skin hook, or forceps to elevate the skin prior to excision of the partial thickness grafts. As many as 70 grafts were taken according to the size of the ulcer to be treated. Large circumferential ulcers were grafted in 2 stages at a week's interval so that sufficient grafts could be obtained. The grafts were evenly distributed over the ulcer with at least 0·5 cm between each to avoid the possibility of epithelial overlap (Fig. 1). The ulcer and donor site were dressed with

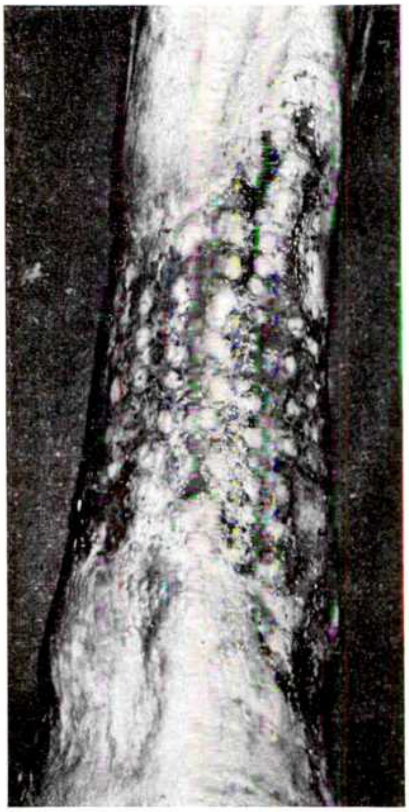

FIGURE 1. Pinch grafts on a gravitational ulcer after 1 week.

Carbonet or Paranet gauze and a firm pad and pressure bandage were applied to each. Patients were allowed to sit out of bed provided that the affected leg was elevated, but walking was discouraged during the first 4 days. The ulcer dressing was changed, if necessary, after 4 days, but the gauze immediately overlying the grafts was left intact for one week. The donor site was dressed on alternate days. Mild infection of some donor sites responded to applications of 1% aqueous gentian violet and all donor sites were healing well at 10 days. The grafts were epithelializing at 7–14 days and healing of the ulcers was well advanced or complete at 21 days.

RESULTS

The success of the initial grafting procedure is shown in Table 1. Twenty-five (75·7%) of the gravitational ulcers healed completely after grafting. Seven of the remaining 8 in this group showed partial take of the grafts in the first instance. In the remaining patient with a gravitational ulcer, two previous attempts at full thickness grafts had failed. Both patients with circumferential ulcers showed complete healing after grafting (Fig. 2). Those patients who showed only partial take or failure of the graft tended to be older than those who had successful grafts.

TABLE 1. Initial success of pinch grafts in gravitational, arteriosclerotic and arteritic ulcers

	Healed		Partial take or failure of graft	
Type of ulcer	No. of ulcers	Age ± s.d. (years)	No. of ulcers	Age ± s.d. (years)
Gravitational	25	60·1 ± 10·3	8	73·2 ± 7.4
Arterio-sclerotic	14	69·0 ± 9.1	—	—
Arteric	2	67·0 ± 5·0	2	52·0

It is noteworthy that all arteriosclerotic ulcers healed initially without any trouble, even though this group, too, tended to be older than those in the gravitational group who had successful grafts. In one patient with Felty's syndrome, and the patient with necrobiosis lipoidica, the ulcers healed slowly. The other patient with Felty's syndrome showed total graft rejection, as did one patient with rheumatoid arthritis. There was no apparent correlation between failure of grafting and the drugs that patients were receiving at the time.

Seventeen of 23 gravitational ulcers which healed after grafting remained intact compared with 10 of the 14 arteriosclerotic ulcers. Four of the 8 successfully grafted gravitational ulcers, which broke down, showed only minimal ulceration and healed within 2 weeks with supportive therapy. None of the grafted arteritic ulcers remained intact. The mean survival of the grafted ulcers in each group, and the mean age of the patients in each group, are shown in Table 2. The mean survival of the grafts in 17 patients with gravitational ulcers, and 10 with arteriosclerotic ulcers, is over 2 years. Surprisingly the survival of the arteriosclerotic group is the longer.

Breakdown of grafts, however, occurs sooner in this latter group at 3·2 months ± 1·8 compared with the 5·7 months ± 1·8 in the gravitational group. Both the surviving grafted arteritic ulcers broke down within 6 months.

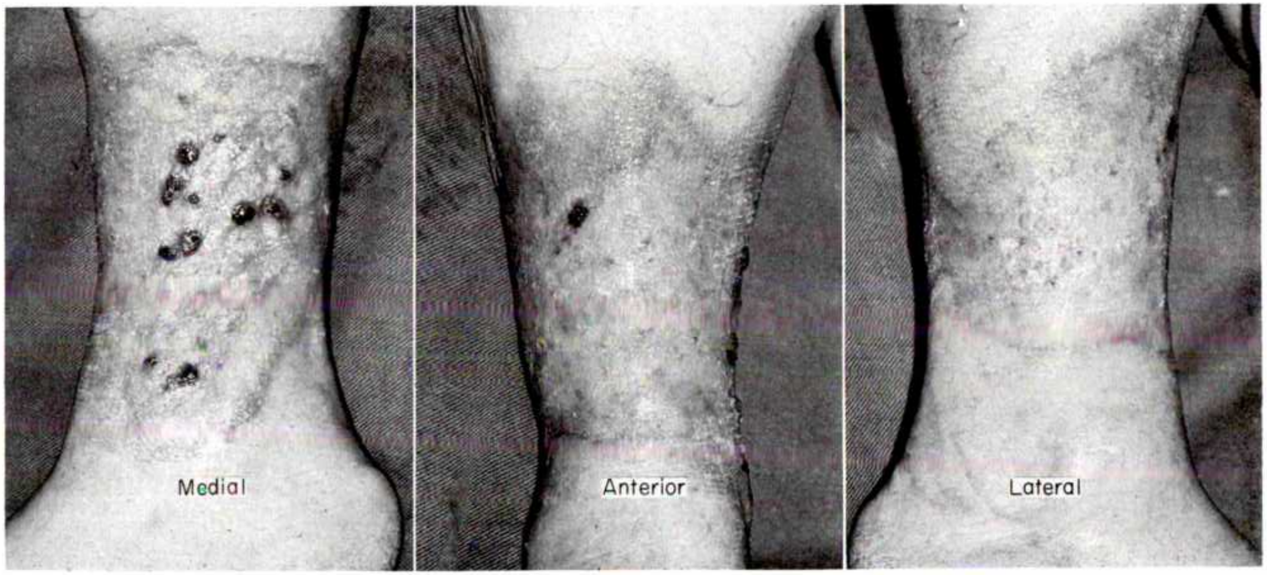

FIGURE 2. Grafted circumferential ulcer showing medial, anterior and lateral surfaces.

TABLE 2. Mean survival of types of grafted ulcers and mean age of patients

Type of ulcer	Grafts remaining intact		Grafts which have broken down		Survival rate (%)
	Mean age of patients (years)	Mean survival of grafts (months)	Mean age of patients (years)	Mean survival of grafts (months)	
Gravitational (25)	61·6±2·8 (17)	24·0±5·0	63·0±4·4 (8)	5·7±1·8	68
Arterio-sclerotic (14)	73·8±2·8 (10)	27·1±5·4	68·5±4·4 (4)	3·2±1·8	71
Arteritic (2)			67·0±5·0 (2)	4·0±0·7	

The number of ulcers is shown in parentheses.

We now use a weekly occlusive dressing on the grafted leg for at least 6 weeks after the ulcer has healed so that the epidermis might be protected whilst it thickens and thus becomes more resistant to breakdown. This appears to be more important in those patients with healed gravitational ulcers because the detrimental effects of postural oedema are also prevented. Where ischaemic ulcers have broken down the new ulcer tended to occur at the side of the graft, rather than in the graft. This new area of ulceration responded to grafting just like the original ulcer.

The survival rate of intact grafted gravitational ulcers was 68% and that of arteriosclerotic ulcers 71%. Expressed as a percentage of the original total grafted, there were 51.5% (17 of 33) gravitational ulcers intact and 71% (10 of 14) of the arteriosclerotic type.

DISCUSSION

Although there have been numerous studies showing the benefits of pinch grafting in accelerating healing of leg ulcers, none has assessed the long term benefits of grafting. This study has shown that the short term gains of early healing are extended to more long term advantages.

The initial success rate after grafting is comparable with others (Reiter, 1948; Chilvers & Freeman, 1969; Smith *et al.*, 1971). The figures correlate very well with other studies (Constantine, Mason & Self, 1969; Smith *et al.*, 1971) which have shown that a high percentage of gravitational ulcers heal within 3 weeks of grafting.

Reiter (1948) grafted 5 ischaemic ulcers unsuccessfully but there have been no other series where this type of ulcer has been pinch grafted.

There are some practical points which need emphasis. No ulcer is too large for grafting and no ulcer need be perfectly clean before it is grafted. Provided that there is no evidence of infection with β-haemolytic streptococcus or heavy contamination with *Pseudomonas pyocyanea*, a granulating ulcer with islands of infected debris can be grafted successfully. We do not give prophylactic systemic antibiotics, but systemic penicillin is always given when there is active streptococcal infection.

The patients are mobilized quickly. Within 24 h they are encouraged to sit out and allowed to walk to the toilet. There appears little advantage in prolonged bed rest as advocated by others (Constantine *et al.*, 1969; Smith *et al.*, 1971). Large ulcers were grafted in 2 or 3 stages. As soon as the first grafts had taken, i.e. become vascularized and pink, further grafts were applied to the remainder of the ulcer. This may occur as soon as 3 days after initial grafting, but to wait until the initial grafted site has become fully epithelialized is to waste time.

Plastic surgeons tend to prefer full thickness whole grafts on leg ulcers because the cosmetic result, both at the grafted and at donor sites, is more acceptable (Gillies & Millard, 1957). We do not share this view. The cosmetic result depends upon the size and the depth of the pinch grafts taken. Other series (Chilvers & Freeman, 1969; Constantine *et al.*, 1969; Smith *et al.*, 1971) used grafts of 0·5–1·5 cm in diameter and sometimes of full thickness (Davis, 1930). We prefer smaller, thinner grafts and, although the procedure then becomes more time consuming, the cosmetic result is very acceptable. Fig. 2 shows a healing circumferential ulcer. The medial side has healed and the lateral side nearly so. The anterior view shows very well the smooth epithelialized surface without the cobblestone appearance said to be the inevitable accompaniment of this type of grafting. The donor sites, too, heal well. Fig. 3 shows a recent healing donor site (A) below an old healed donor site (B) from which grafts had been taken 6 months previously. The cosmetic result is not disfiguring and, after this length of time, barely noticeable. In 2 patients, however, the initial site of application of the grafts was obvious 2 years later (Fig. 4).

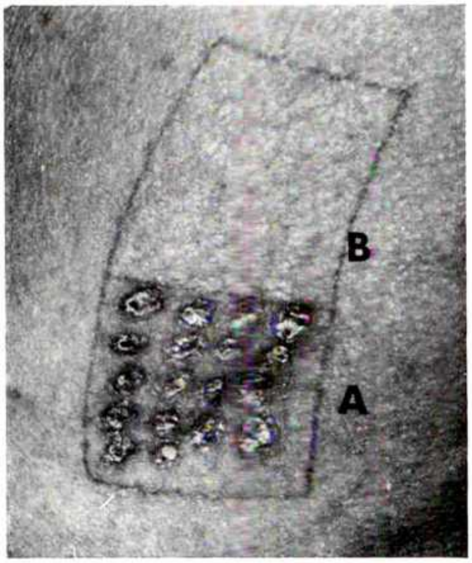

FIGURE 3. Donor sites (A) recent; (B) 6 months old.

Pinch grafting is an old technique, but this present study shows that it still has much to recommend it in the management of chronic gravitational and arteriosclerotic leg ulceration. Early treatment by grafting has reduced the frequency of out-patient attendances for these patients and shortened their in-patient stay. This simple procedure has accelerated healing and reduced subsequent morbidity in the long term even though the underlying venous or arterial disease could not be surgically treated. We feel that, if possible, all in-patients should be offered this form of therapy.

Our initial experience suggests that out-patients, too, may benefit from pinch grafting. This technique can be adapted to their special needs although the patients need to be selected more rigorously because a greater degree of co-operation is needed. In particular they must take great care to maintain the stability of the dressings over the grafts. There is reason to believe, however, that comparable success rates should be achieved with out-patients as we have shown with in-patients.

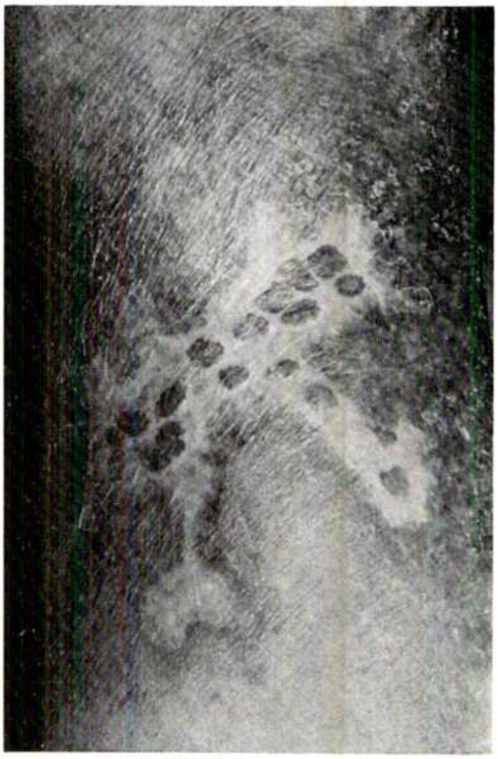

FIGURE 4. Grafted ulcer showing persistent pattern of grafts after 2 years.

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Psoriasis and stress*

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SUMMARY

132 psoriasis patients who had been completely cleared with dithranol were assessed and then followed up over a 3-year period. The incubation time from specific incidents of stress to the development of psoriasis was between 2 days and 1 month. Specific stress within a month before the first attack was recalled by 51 patients (39%); chance association was excluded by studying a control group where there was significantly less specific stress. The prognosis for the psoriasis patients who recollected specific stress a month prior to the onset was significantly better than for the rest of the patients where none had been recalled.

That there is a relationship between mind and matter has been observed by physicians and philosophers certainly from the time of Socrates. He reported that the physicians of Thrace were ahead of the Greeks, as they knew the body could not be cured without the mind; 'and this was the reason why most maladies evaded the physicians of Greece—that they neglected the whole . . .' (Plato *c.* 390 B.C.).

Since that era there has been much clinical observation and anecdotal writing on the subject and dermatologists have contributed considerably to both. Sceptics have, on the other hand, pointed out that psychological stress is present in most patients' lives and that a relationship between it and illness has not been proven.

It was therefore decided to study patients suffering from psoriasis, as a series was going to be available where the treatment would be standardized. Also it was thought that any observation throwing light on possible causes of chronicity would be valuable in a condition still thought by some to carry a heavy life sentence.

MATERIALS AND METHOD

183 consecutive non-pustular, non-exfoliative, non-guttate psoriatic in-patients were studied personally. Dithranol treatment had been kept constant over a 3-year period, apart from deleting tar from the bath and stopping the ultraviolet light halfway through the experiment. This change did not significantly affect treatment times or the relapse rate (Seville, 1975), virtually equal results being obtained after subsequent allowance for stress. Complete clearance was the only criterion for cessation of treatment with the exception of the 14 patients mentioned later. A full general and psychological

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* Based on a paper given to the Dowling Club at the Pahlavi Congress, Shiraz, April 1975.

history was taken on admission and recorded personally on a proforma from which punch cards were ultimately prepared. A standard question 'What upset or illness was there just before the onset of the rash?' was asked to ascertain if there had been specific stress before the onset of the eruption. Any reactions to the question, the degree of response and other nuances were noted at the time.

Specific stress was defined to include only major upsets as experienced by the patient. The detailed histories on the proformas were checked by an independent medical observer (G.B.W.) who agreed that the stress had been specific. The time from upset to the attack was carefully checked and is referred to in this article as the incubation time. Practitioner records were consulted when necessary. The unprefaced word 'clear' is defined to include also those patients where full examination revealed the occasional minute lesion or patch of roughness in the scalp of which the patient was often unaware.

The following patients had to be excluded from the series: 7 discharged early in the treatment for family reasons but not clear, 7 treatment failures, 8 deaths, 17 who left the district and were lost to follow-up, and 12 where the original attack had followed within a month of intercurrent infection. This left 132 patients completely cleared with dithranol out of the original 183. Careful personal follow-up was then undertaken up to a period of three years and the time of relapse established by careful questioning.

Incubation time

Relapses of psoriasis were added in this section to first attacks in order to give a greater number of instances and with more accurate memory for recent events.

The time interval between specific incidents of stress and attacks could be remembered actually in days or weeks as follows:

Intervals in days	2	3	5	6	7	8	10	14
(38) Instances	8	4	2	1	10	1	6	6

Mean 7 days, mode 7 days, median 7 days.

Interval in weeks	1	1½	2	2½	3	3½
(54) Instances	17	3	19	4	9	2

Mean 1·6 weeks, mode 2 weeks, median 2 weeks.

The number of instances remembered within one month was 19 and at 1 month was 6. The total number of instances was 117. Excluded were 5 (4%) incidents as the time was more than one month.

Comment

The incubation time for specific incidents of stress and the development of psoriasis as noticed and remembered by the patient lay between 2 days and 4 weeks in 96% of the instances, the range being virtually the same as that described by Bolgert (1957). A period of one month was, therefore, adopted to see if the stress was a chance association and if there were differences in the prognosis.

Stress factors

Specific incidents of stress within 1 month before the first attack of the psoriasis were recalled by 51 patients; the incidents were found to fall under the following headings: major interpersonal upsets within the family group (21), deaths or hospitalization of close relatives (16), examinations (10), accidents (3) and sexual assault (1). One patient developed psoriasis with the 11+ examination, and relapsed with 'O' levels, 'A' levels and university finals.

Control series

A careful enquiry, as in the psoriasis series, was made to find out what number of patients with conditions not thought to be psychiatrically determined, suffered similar specific stress by chance within a month of their attendance. One group with upper respiratory and other infections was seen by a senior clinical assistant (G.B.W.) at his surgery and another group with benign or malignant skin tumours attending dermatology out-patients was assessed personally. Recent memory would give a higher figure than in the psoriasis series where the questioning concerned the first attack which was often some time ago; also the patients were well-known to the general practitioner who would have family knowledge of any major upsets. The general practice group was separately matched for age and sex with the 132 psoriasis patients.

It was found that 13 (10%) had recalled specific stress within a month of their attendance, whereas 51 patients (39%) recollected specific stress before the first attack of psoriasis. The difference showed a high significance level of 5·4 times the standard error (s.e.), $P<0{\cdot}001$. Four (5%) out of 73 patients with benign or malignant tumours recalled specific stress within a month of their attendance. The level of significance was 4·9 × s.e. ($P<0{\cdot}001$), when compared with the psoriasis series. These findings indicate that the effect of specific stress was not a chance association in the precipitation of psoriasis.

PROGNOSIS

Many factors, some interrelated, affect the prognosis in psoriasis and all were cross checked separately. Those where the variation was less than 5% are detailed as follows:

Whole series	Number	132	Clear at 3 years 41 (31)*
Age at first attack	Under 15 years	58	17 (29)
	Over 15 years	74	24 (32)
Family history	With	53	16 (30)
	Without	79	25 (32)
Sex	Male	52	15 (29)
	Female	80	26 (33)

* Percentage in parentheses.

It can be seen that there is agreement with the findings of the following writers: after clearance with dithranol, there was no difference in the relapse rate with the presence or absence of family history, or age at onset (Ingram, 1954; Durham & Morgan, 1974), or age at treatment time (Rossi-Soffar, 1965).

As family history has been considered an indicator for tendency to psoriasis, the 51 patients who had recollected stress before the first attack were examined further: 23 had a positive family history and of these 12 (52%) were clear at 3 years; when the family history was negative there were 28 patients and 15 were clear 3 years later giving a virtually identical figure of 54%. This will be referred to later in the discussion.

Factors of prognostic importance in the 132 patients are as follows:

		No.	Clear at 3 years
Time from onset to consultation and treatment	Within 1½ years	66	25 (38)*
	Over 1½ years	66	16 (24)
Stress recollected within one month prior to the onset		51	27 (53)
No stress recollected		81	14 (17)
Condition on first admission	Mild	25	10 (40)
	Moderate	79	26 (33)
	Severe	28	5 (18)

* Percentage in parentheses.

It can be seen that the relapse rate is lower when the time between onset and consultation and treatment had been short (Ingram, 1954; Romanus, 1945), when the rash was not severe and when the rash had followed stress; these factors were therefore cross-checked by pairing the patients as will be described in the next section.

Severe involvement was commoner when psoriasis began in childhood (Farber & Carlsen, 1966; Church, 1958) but it was duration and not the age at onset that was important in the present series; in any event severe cases were excluded in the pairing.

Specific stress results

There were 51 patients (39%) who recollected specific stress occurring within a month prior to the first attack. The relapses of these patients were compared with the 81 comprising the rest of the series as shown in Fig. 1.

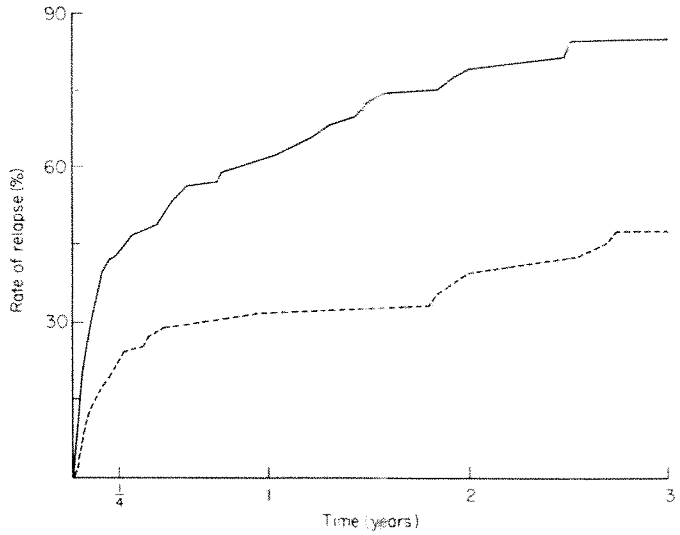

FIGURE 1. Relapses of 51 patients with prior recollected stress (- - -) (17% clear after 3 years), and 81 where none had been recalled (——) (53% clear after 3 years).

Pairing was then undertaken to exclude the variables in the series mentioned in the paragraph on prognosis and firstly 28 patients with severe psoriasis on admission were excluded:

		Clear at ¼ year	Clear at 1 year	Clear at 3 years
With prior stress	41	33 (80)*	31 (76)	24 (59)
Without stress	63	39 (62)	28 (44)	12 (19)
Significance		2 × s.e. $P = 0{\cdot}046$	3·1 × s.e. $P < 0{\cdot}0027$	4·05 × s.e. $P < 0{\cdot}001$

* Percentage in parentheses.

To exclude the other variable in the series, patients were then studied where the time between the first attack of psoriasis and consultation and treatment was under 1½ years:

		Clear at ¼ year	Clear at 1 year	Clear at 3 years
With prior stress	26	25 (96)*	24 (92)	17 (65)
Without stress	29	16 (55)	12 (41)	5 (17)
Significance		3·1 × s.e. $P < 0{\cdot}0027$	3·9 × s.e. $P < 0{\cdot}001$	3·6 × s.e. $P < 0{\cdot}001$

* Percentage in parentheses.

Statistical note: (T.W. & G.B.W.) The figures were calculated from the standard error of the difference of the two proportions. The numbers of patients in the last table were slightly below 30 but the levels of significance were so high that they were considered significant.

Included in the 81 patients with no recollected stress before the first attack were 7 who, after they had relapsed and were therefore out of the survey period, were able to recall the original upset at a subsequent consultation. If these 7 patients are added to the 51, it gives a figure of 44% with stress before the first attack.

CONCLUSION

It can be seen that when specific stress within a month before the onset had been recollected, the relapses were less than when no stress had been discovered. The reduction of relapse rate was demonstrated to be significant after exclusion of severely affected patients, and when consultation and treatment had been early.

DISCUSSION

In the first Watson Smith lecture, Ingram (1954) considered emotional stress the most potent precipitating factor in psoriasis and that it was amenable to the 'constitutional approach'. In the present series first attacks of psoriasis followed within 1 month, and were related to specific stress in 44% of the patients studied. Bolgert (1957) reported that outbreaks of psoriasis followed 'provocative psychological factors' in 48% and probably also in a further 20·5% of all his patients. Many writers agree that nervous factors can adversely affect psoriasis. Their figures range from 90% (in children, Nyfors

& Lemholt, 1975), 70% (Susskind & McGuire, 1959), 57% (Wittkower & Russell, 1953), 53% (Hellgren, 1964), 50% (Savin, 1970), 40% (Farber, Bright & Nall, 1969), to more than 15% (Church, 1958), and less than 10% (Lomholt, 1963). Baughman & Sobel (1971) showed that psoriasis varied with stress to a moderate but significant degree and Coles & Ryan (1975) advocated psychotherapy in its treatment. Bolgert (1971) and Goldsmith, Fisher & Wacks (1969) noted an increased incidence of psychiatric disorders in psoriatics and recommended a combined dermatological and psychiatric therapeutic approach and the use of tranquillizers.

No explanation has been given as to why the prognosis is better when psoriasis has followed stress. All patients were certainly given the same encouragement and a feeling that the staff really cared. Was stress required as a precipitating factor for the rash to develop in patients with less tendency to psoriasis? Family history may be considered an indicator of tendency but it has already been shown that the percentage of patients clear at 3 years was similar whether a family history was present or not in the stress group, or in the whole series.

The better prognosis would appear clinically to be due to the presence of insight; 'know thyself' was indeed the essence of the teaching of Socrates.

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Cutaneous β-glucuronidase: cleavage of mycophenolic acid by preparations of mouse skin

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SUMMARY

Mycophenolic acid, a new chemotherapeutic agent for the treatment of psoriasis, is known to be rapidly conjugated on absorption and to circulate largely in the form of its glucuronide conjugate. Since this metabolite does not readily penetrate intact cells and is cleaved by the enzyme β-glucuronidase to yield free mycophenolic acid, the ability of preparations of mouse skin to cleave mycophenolic glucuronide was studied. The time course of mycophenolic acid liberation by such preparations and the dependence upon the amount of enzyme preparation were demonstrated. Preparations of mouse skin and mouse liver, kidney, spleen, lung, heart and small intestine were assayed for β-glucuronidase activity using *p*-nitrophenyl-β-D-glucuronide as substrate. Skin yielded preparations with higher β-glucuronidase activity per/mg protein than any of the other organs tested. When expressed on the basis of β-glucuronidase activity recovered per milligram of tissue DNA, skin, liver and kidney showed higher levels than the other organs tested.

Mycophenolic acid (MPA), an inhibitor of guanosine-5-monophosphate (GMP) synthesis, has been shown in a pilot clinical study to be effective in the treatment of severe psoriasis when administered orally in high dosage (Jones *et al.*, 1975). This drug has previously been shown to have a variable chemotherapeutic value in treating tumours in experimental animals (Sweeney *et al.*, 1972a). Sweeney *et al.* (1971) have also shown that the effectiveness of MPA in inhibiting tumour growth is dependent upon the presence of high levels of β-glucuronidase activity in the tumours. This paper reports the cleavage of mycophenolic acid-β-D-glucuronide (MPAG) to MPA by homogenates of mouse skin and compares the β-glucuronidase activity of various organs.

MATERIALS AND METHODS

Materials

Mycophenolic acid and mycophenolic acid-β-D-glucuronide were supplied by Eli Lilly and Company. Reagents for β-glucuronidase assay were purchased in prepared form from Sigma Chemical Company. Reagents and standard solutions were stored at 4°C.

Preparation of homogenates

Eight-week-old male 0618 HRS/J mice were killed by cervical dislocation and decapitation. Skin and other organs were promptly excised, chilled, weighed and frozen in liquid nitrogen. Organs to be assayed were minced with scissors while in cold distilled water (4°C) sufficient to yield a 10% homogenate, and then homogenized in succession with a polytron and a teflon glass homogenizer while chilled in an ice bath.

The homogenate was centrifuged at 600 *g* for 10 min at 4°C to remove unbroken cells and debris and the supernatant was further centrifuged at 10,000 *g* for 20 min. The 10,000 *g* supernatant was either used immediately for enzyme assays and protein determination, or frozen at −20°C.

Assay of mycophenolic acid-β-D-glucuronide cleavage

A stock solution of MPAG, having a concentration of 0·2 mg/ml (0·403 mM), was prepared in 0·05 M acetate buffer (pH 4·5). Reaction mixtures containing 0·4 ml MPAG stock solution and 0·1 ml of the enzyme preparation to be assayed were incubated at 37°C for 30 min and the reaction stopped by addition of 9·5 ml of 0·1 M borate buffer (pH 10·1). The amount of free MPA formed was then determined by fluorescence using an excitation wavelength of 348 nm and emission setting of 430 nm. Fluorescence was compared to MPA standards in 0·1 M borate buffer.

Assay of p-nitrophenyl-β-D-glucuronide cleavage

The enzyme preparation (0·1 ml) was mixed with 0·1 ml of 60 mM *p*-nitrophenyl-β-glucuronide (PNPG) in 0·2 M acetate buffer (pH 4·5) and 0·3 ml H_2O and incubated for 1 h at 37°C. The reaction was stopped by addition of 2·5 ml of 0·1 M glycine buffer (pH 10·5) and the *p*-nitrophenol liberated determined by absorption at 400 nm.

DNA and protein determinations

DNA content of tissues was determined by the method of Burton (1956). The protein in enzyme preparations was determined by the method of Lowry *et al.* (1951).

RESULTS

Cleavage of MPAG

The 10,000 *g* supernatant of mouse skin homogenate was incubated with MPAG for varying periods of time. As shown in Table 1, MPAG was readily cleaved to free MPA. Under these reaction conditions the rate of cleavage was linear for at least 45 min. Subsequent experiments used 30 min incubations. The dependence of the reaction on the skin extract is shown in Table 2.

TABLE 1. Time course of MPAG cleavage by 10,000 *g* supernatant of mouse skin preparations

Time (min)	nmol of MPA liberated/mg tissue*
0	0
10	2·08
20	4·17
30	6·25
45	9·37
60	11·46

* Average of duplicate determinations.

TABLE 2. Dependence of MPAG on 10,000 *g* supernatant of mouse skin preparations

Supernatant (μg of protein)	nmol of MPA liberated*
0	0
60	11·2
120	25·6
180	38·5
240	48·9
300	56·7

* Average of duplicate determinations.

β-Glucuronidase activity of mouse organs

Because of the limited availability of MPAG, comparison of β-glucuronidase activity in various organs was carried out using the colorimetric substrate, *p*-nitrophenyl-β-D-glucuronide. Homogenates of skin, liver, lung, kidney, spleen, heart and small intestine were prepared and the 10,000/*g* supernatants assayed for β-glucuronidase activity and protein concentration. In addition, the DNA content of these organs was determined. Table 3 shows the β-glucuronidase activity in the organ homogenates, expressed both as activity/mg of protein in the supernatant and as activity/mg of DNA in an equivalent amount of tissue from that organ. When expressed as activity/mg of protein, the skin supernatant contained higher activity than any of the other organs assayed. When expressed in relation to the amount of DNA, which is a measure of the tissue cellularity, the β-glucuronidase activity of skin was exceeded only by that of liver and kidney, and was considerably above that for spleen, lung, heart and small intestine.

TABLE 3. Tissue β-glucuronidase

Tissue	Activity/mg protein*	Activity/mg tissue† DNA
Skin	0·41 ± 0·10 (13)	5·8
Liver	0·19 ± 0·04 (8)	7·3
Kidney	0·20 ± 0·06 (8)	7·5
Spleen	0·21 ± 0·06 (8)	1·2
Lung	0·07 ± 0·04 (8)	2·2
Heart	0·01 ± 0·02 (8)	0·5
Small intestine	0·11 ± 0·05 (6)	1·1

* μmol of *p*-nitrophenol produced/h/mg of protein ± standard deviation. Numbers in parentheses are the number of animals studied.

† Average μmol of *p*-nitrophenol produced/h/mg of DNA in an equivalent amount of tissue.

DISCUSSION

In cell free systems, both mycophenolic acid (MPA) and its glucuronide conjugate (MPAG) are potent inhibitors of IMP dehydrogenase and GMP synthetase (Sweeney *et al.*, 1972b). In systems having intact cells the activity of MPAG is less than that of MPA and is highly variable, since MPAG does not readily enter cells and must be cleaved to free MPA, which readily penetrates cells and inhibits

GMP synthesis. Sweeney *et al.* (1971) found a close correlation between β-glucuronidase activity of a number of tumours and the inhibition of tumour growth by systemically administered MPA. Systemically administered MPA is rapidly conjugated by the liver to form MPAG, which is the major circulating metabolite and the major excretory product (Lintrup *et al.*, 1972). As a result, systemically administered MPA has low toxicity except for the gastrointestinal tract which is exposed to orally administered drug prior to conjugation.

Cleavage of β-glucuronides by tissues is generally accepted to be catalyzed by an acid hydrolase, β-glucuronidase, which is specific for the glucuronide moiety and relatively nonspecific as to the rest of the molecule, acting on a wide range of naturally occurring and synthetic glucuronide compounds.

The finding that orally administered MPA is effective in the treatment of psoriasis (Jones *et al.*, 1975) suggests that skin may have considerable ability to cleave the circulating MPAG to free MPA. Ohkawara *et al.* (1972) studied acid hydrolases in human epidermis and found levels of β-glucuronidase which liberated approximately 0·05 μmol of PNP/h/mg of protein. More recently, Mier and van den Hurk (1975) have studied the β-glucuronidase of guinea-pig epidermis, using 4-methylumbelliferyl-β-D-glucuronide as substrate.

Because one of the major areas of interest in the pharmacology of MPA is the difference in toxicity and effect in various organs, we have studied the distribution of β-glucuronidase in various organs of the hairless mouse and the ability of mouse skin preparations to cleave MPAG. Mouse skin preparations readily cleaved MPAG to free MPA and also yielded preparations with higher β-glucuronidase activity/mg protein than other organs. Even when corrected for the cellularity of the various tissues by correcting for tissue DNA, skin was close to the values obtained with liver and kidney and significantly higher than spleen, lung, heart and small intestine. Since MPA has been found to be effective in the treatment of psoriasis, the finding of significant β-glucuronidase in skin is consistent with the pattern noted with experimental tumours (Sweeney *et al.*, 1972a). The values obtained with whole mouse skin using PNP as substrate are approximately 8 fold higher than found by Ohkawara *et al.* (1972) in human epidermis. Since it is the relative tissue levels of β-glucuronidase in various organs that are of interest, it is not possible to apply our data, obtained with mice, to the clinical situation. In particular, psoriatic skin may well have levels of β-glucuronidase that differ significantly from normal skin. Also, factors other than β-glucuronidase, such as the presence of an active salvage pathway for guanine, may be of importance in determining the effect of MPA on GMP synthesis.

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Post-kala-azar dermal leishmaniasis

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SUMMARY

Ten patients with post-kala-azar dermal leishmaniasis (2 females, 8 males—aged 17–47 years) are described. Two of them had macular lesions in which very few Leishman–Donovan bodies were seen and who responded well to treatment with stibophen. Five patients had both macular and nodular lesions, Leishman–Donovan bodies being present in nodules but not in macules. Treatment with stibophen caused partial regression of nodules.

Nodular lesions were found in three patients, who did not have macules. They contained numerous Leishman–Donovan bodies and treatment produced only temporary improvement.

The variable response to treatment with stibophen may have been due to differences in parasitic load. The difficulty in distinguishing this disease from leprosy is discussed.

Kala-azar (visceral leishmaniasis) caused by *L. donovani* and transmitted by the sandfly is endemic in many tropical countries. Cutaneous involvement, post-kala-azar dermal leishmaniasis (PKDL), has been reported in about 6% of cases, occurring 1–5 years after the original infection (Napier & Das Gupta, 1930; Manson-Bahr, 1966).

Firm nodules around the mouth and nose are characteristic of the disease but may also occur on the trunk and limbs. Hypopigmented macules may precede or coincide with the onset of nodules and are particularly prominent on the back. A 'butterfly' erythema on the face, aggravated by sunlight, is often the earliest sign.

Although attempts to eradicate the sandfly have led to PKDL becoming increasingly rare in some countries (Majumdar, 1967; Yesudian & Thambiah, 1974), the occasional case is encountered throughout the world (Jopling, 1977) and dermatologists should be aware of the disease. We therefore describe 10 cases of PKDL, seen at the Banaras skin clinic in the past 6 years. Like Majumdar (1967), we found that patients could conveniently be divided into three clinical groups.

CASE REPORTS

Group A. Cases with macular lesions only

Case 1. A 24-year-old female from Nepal with hypopigmented macular lesions on the upper back,

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sides of the neck and upper arms present for 4 years. A coppery-purple discolouration was present on the 'butterfly' area of the face, which became worse on exposure to sunlight. She had been treated for kala-azar 1 year before the onset of the rash. General examination was normal. Sensation in the skin lesions was normal and there was no evidence of peripheral nerve involvement.

Acid-fast bacilli were not found on slit-smear examination of lesions. Biopsy of a hypopigmented area revealed dense linear infiltration of the upper dermis by lymphocytes, plasma cells and histiocytes. A few intracellular Leishman–Donovan bodies were found after a prolonged search.

The patient was treated with 25 injections of stibophen (Fantorin-Glaxo, 1 ml equivalent to 8·6 mg of antimony) 2 ml intramuscularly on alternate days. The same treatment was given twice more with a three week interval between each course. No new lesions were seen during 3 years of follow-up; some disappeared after approximately 1 year. A further skin biopsy was taken from a persisting macule 1 year after treatment and showed a patchy, mainly lymphocytic, infiltrate in the upper dermis. No Leishman–Donovan bodies could be seen.

Case 2. A 30-year-old female with multiple hyperpigmented macules interspersed with hypopigmented areas on the trunk, gluteal regions and thighs present for 11 years. The lesions had developed one year after treatment for kala-azar. Biopsy findings were similar to those seen in Case 1. The patient was also treated with stibophen in the same manner and showed slow improvement. No new lesions were seen during 2 years of follow-up.

Group B. Cases with macules and nodular lesions

Cases 3–7 consisted of five male patients who were local weavers, aged 17–29 years with skin lesions present for 3–7 years. They gave a history of kala-azar 1–3 years before the onset of the rash.

A coppery erythematous macular eruption on the cheeks was usually noted first followed by small firm nodules below the lower lip, on the sides of the nose and in the circumoral region. New lesions developed gradually on the neck, axillae, dorsal surfaces of the hands and feet, and antecubital fossae (Figs 1–2). Prominent hyperpigmented linear macules were also present in the axillae and gluteal regions (Fig. 3). Physical examination was otherwise normal and slit-smear examinations for acid-fast bacilli were negative.

Histology of macular areas showed increased pigmentation in the basal layer of the epidermis. A mixed chronic inflammatory cell infiltrate was present in the upper dermis; Leishman–Donovan bodies were not seen. Nodular lesions consisted mainly of histiocytes containing Leishman–Donovan bodies with plasma cells, lymphocytes and occasional giant cells. The infiltrate was present in the middle and upper dermis, encroaching upon and stretching the epidermis.

The patients were treated, as previously described, with regression of nodular lesions. At the end of treatment, however, these lesions enlarged but not to their original size. Biopsies were taken from some of the patients after treatment and although a chronic granulomatous reaction was present, Leishman–Donovan bodies could not be seen.

Group C. Cases with nodular lesions only

Cases 8–10 consisted of three patients, aged 30–47 years, with skin lesions present for 1–8 years, who were referred as suspected cases of leprosy. Firm nodules of various sizes were present around the mouth, nose and scattered on the trunk and limbs (Fig. 4). Macular lesions were not seen. There was no other evidence of kala-azar or leprosy on physical examination. A history suggestive of kala-azar 3–17 years prior to the development of lesions was elicited in all of them.

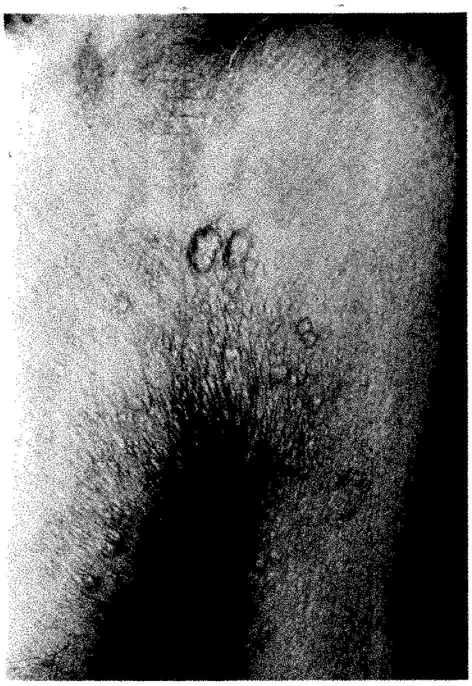


FIGURE 1. Granulomatous nodular lesions in the anterior axillary fold.

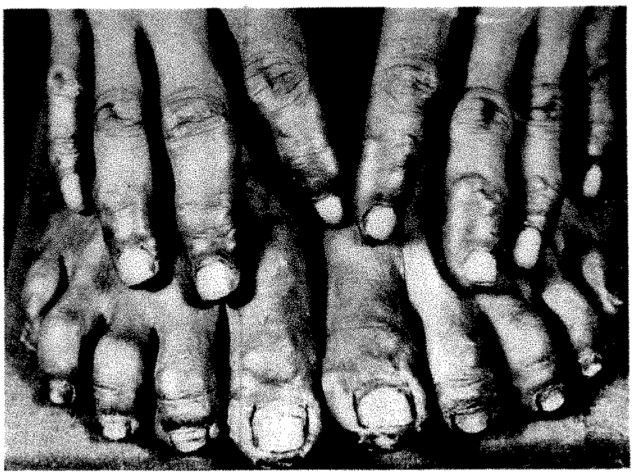
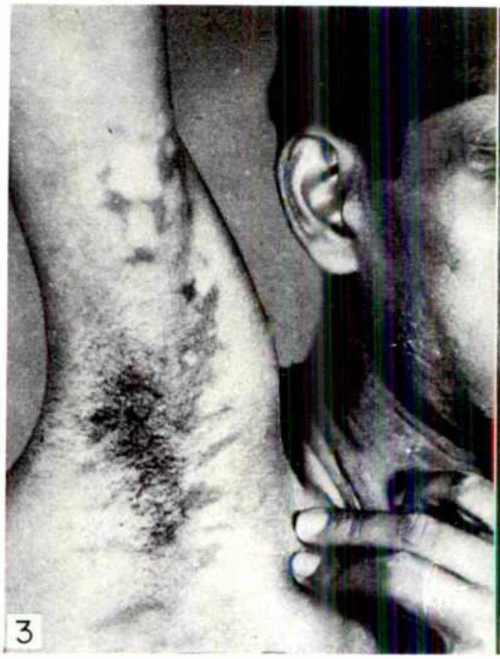


FIGURE 2. Nodular infiltrations on the dorsal surfaces of the knuckles of both hands and feet.

On histological examination the epidermis was flattened and the upper dermis relatively free of cells. The mid and lower dermis was heavily infiltrated with histiocytes containing large numbers of Leishman–Donovan bodies. Plasma cells and lymphocytes were also present.

The patients were treated with stibophen as described earlier and remission occurred quickly. On withdrawing the drug, however, there was immediate relapse.



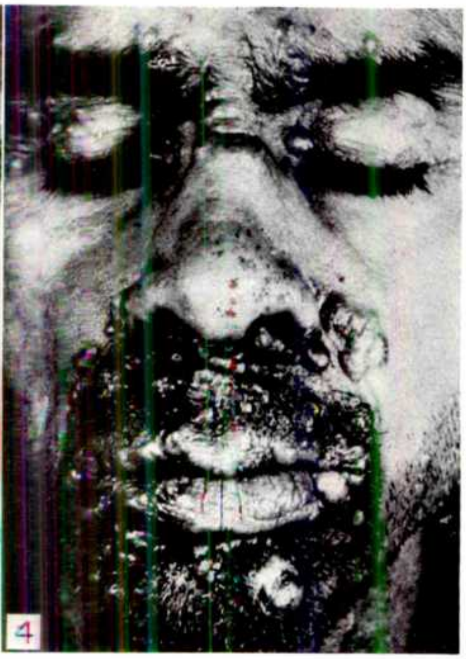


FIGURE 3. Hyperpigmented macules in the axillary fold.

FIGURE 4. Firm granulomatous lesions around the mouth, nose and frontal region with intact eyebrows.

DISCUSSION

The macules and nodules of PKDL resemble those found in leprosy, and consequently problems in diagnosis may occur (Munro, Du Vivier & Jopling, 1972). Our difficulty, at the Banaras clinic, is to identify a small number of patients with PKDL from among 4000 new cases of leprosy seen each year. Because of their multiple nodules the patients in Group C were thought to have lepromatous leprosy and PKDL was not suspected until slit-smear examinations failed to demonstrate lepra bacilli. The co-existence of macules and nodules in Group B, macules usually preceding nodules, was more suggestive of borderline leprosy, whereas the two patients in Group A had hypopigmented macules similar to maculo-anaesthetic lesions of tuberculoid leprosy (Indian Leprologists Classification, 1964).

PKDL can be distinguished from leprosy by the presence of normal sensation in skin lesions and lack of peripheral nerve involvement. The absence of acid-fast bacilli on slit-smear examination of nodules rules out a diagnosis of leprosy. A history of preceding kala-azar, which was helpful in our cases, cannot always be obtained (Majumdar, 1967).

Leishmaniasis was confirmed histologically, in all our cases, by the demonstration of Leishman–Donovan bodies. The number of organisms seen varied however; in macules they were infrequent and difficult to find. Leishman–Donovan bodies were more numerous in nodules from Group B, being present in most high power fields. Large numbers were found in lesions from Group C.

Parasitic load may determine the response of cutaneous leishmaniasis to treatment (Ercoli, 1966) and could explain our findings. Patients in Group A with relatively mild disease and few organisms in their lesions responded well to therapy. In contrast, those in Group C had many nodules containing large numbers of Leishman–Donovan bodies. Such patients showed a greater tendency to relapse after treatment. Perhaps a spectrum of immunological activity might exist in PKDL similar to that described in other forms of cutaneous leishmaniasis (Bryceson, 1970, 1972). It is of interest that the males in our series were more severely affected than females.

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Evolution of the lesion of discoid lupus erythematosus: a comparison with lichen planus

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SUMMARY

The tissue processes leading to the development of the epidermal changes in the lesion of discoid lupus erythematosus have been studied. Typical lesions in fifteen patients have been examined using cell kinetic and immunofluorescence techniques. The results suggest that a very active repair mechanism is operative within the epidermis with heightened epidermopoiesis and epidermal cell migration. The findings have been contrasted with those of previous similar studies of lichen planus. It is suggested that a major difference between the gross and microscopic appearance in the two diseases may be partially on the basis of differing patterns of immunoprotein deposition.

At times there is a close similarity between the histological appearances of discoid lupus erythematosus (LE) and lichen planus (LP) (Copeman, Schroeter & Kierland, 1970; Thormann, 1974; Davies *et al.*, 1977). We have been prompted by these morphological resemblances to ask whether the diseases share important pathophysiological mechanisms. Previously, studies of LP by our group have revealed that the epidermal hypertrophy and hyperkeratosis may be regarded as a consequence of persistent damage to the epidermal generative compartment and attempts at its reparation (Marks, Black & Wilson Jones, 1973; Presbury & Marks, 1974). Here we describe similar studies in discoid LE and draw conclusions that we believe have exciting implications for both diseases.

METHODS

Histological and autoradiographic techniques

Twenty-one lesions from 15 patients with clinically and histologically typical discoid LE were injected intracutaneously with 10 μCi of tritiated thymidine (^{3}H Tdr) (Radiochemicals, Amersham, specific activity 26 Ci/mmol) in 0·1 ml of normal saline. The age and sex of the patients and the site and duration of the lesions are given in Table 1. Twelve of the biopsies were removed 1 h after injection and the remaining 9 were removed at differing times within the subsequent 7 days (Fig. 1). Biopsies were fixed in 10% formol saline, dehydrated, wax embedded and sectioned according to routine methods. Sections were dewaxed and prepared autoradiographically using the dipping film method of Joftes & Warren (1955) and Ilford K2 nuclear emulsion. They were developed in Kodak D19 developer and stained through the emulsion with haematoxylin and eosin. Sections from all biopsies were also stained with periodic acid Schiff reagent to show the basement membrane (Stough-

TABLE 1. Age, sex and biopsy details of the patients

Patient	Age (years)	Sex	Biopsy details		
			Investigations	Site	Duration of lesion (years)
1	60	F	1 h LI	Forehead	5
2	50	F	1 h LI	Forehead	2
3	60	F	1 h LI	(L) Cheek	7
4	51	F	1 h LI	Neck	3
5	37	F	1 h LI	Neck	2
			Interval L	Forehead	3
6	34	M	1 h LI	(L) Temple	1
7	32	F	1 h LI	Upper chest	6/12
8	32	M	1 h LI	Angle of Jaw	14
			Interval L	Angle of Jaw	14
9	66	F	1 h LI	(L) Cheek	10
			Interval L	(R) Cheek	10
10	63	F	1 h LI	(R) Forearm	9/12
			Interval L.	(R) Forearm)	9/12
11	55	M	1 h LI	Neck	8/12
			Interval L	Neck	8/12
12	66	F	1 h LI	Back of (L) hand	4/12
			Interval L	(L) Forearm	4/12
13	44	F	Interval L	Nose	9/12
14	40	F	Interval L	(R) Cheek	2
15	46	F	Interval L	Neck	3/12

LI, labelling index; Interval L, interval labelling.

ton & Wells, 1950). The autoradiographs and PAS stained sections were examined microscopically and the overall morphology of the epidermis, the disposition of autoradiographically labelled cells and the breadth of the PAS staining basement membrane were graphically recorded using a drawing tube. Labelling indices (LI) were calculated for the autoradiographs derived from skin injected 1 h prior to removal with tritiated thymidine ('1 h ARs') by counting the number of labelled basal and suprabasal cells in 1000 interfollicular basal cells. The paralesional skin included in these biopsies was also inspected for labelled cells and an 'uninvolved LI' was calculated for these areas in 6 patients.

Immunofluorescence technique

Portions of 16 of the biopsies were quench fixed in a freezing mixture of cardice and hexane and after storage in liquid nitrogen sectioned on a cryostat at 10 μm. These sections were examined for the presence of IgG, IgM, IgA, complement (C3) and fibrin by the immunofluorescent method using the direct technique with a Nikon SKT microscope fitted with epi-illumination (12 V, 50 W Tungsten halogen light source with interference blue filter, KP 5000). The reagents used were as follows:

Fluorescein conjugated sheep antihuman antisera to IgG, IgA, IgM (Wellcome), rabbit anti-serum to human fibrin and goat anti-serum to human C3 (Hyland). Dilutions of antisera used were as follows:

anti IgG 1/30, anti IgA 1/10, anti IgM 1/20, antifibrin 1/30, anti C3 1/30.

In vitro *studies of epidermal migration*

Fragments of typical lesions from 20 patients were cut into explants approximately 4 mm long, 4 mm wide and 2 mm thick. These explants were then placed epidermis upwards in Eagle's minimal essential medium supplemented by 10% serum in plastic disposable petri dishes. The dishes with explants were then incubated at 37°C for 24 h periods in 95% air and 5% CO_2. At the end of incubation the explants were removed, fixed in formalin and then prepared histologically. The resulting sections were evaluated for the amount of epidermis that had migrated from the edges of the explant and from the damaged areas of the dermo-epidermal junction.

RESULTS

Autoradiographic findings

In the '1 h ARs', labelled epidermal cells were seen in the basal and suprabasal layers and the overall distribution of these labelled cells was surprisingly uniform in the discoid LE plaques. Labelled keratinocytes were found alongside degenerating cells in the involved areas (Fig. 2). The external root sheaths of the follicles showed a similar distribution and density of labelled cells. The mean labelling index in the involved areas for the '1 h ARs' was 21·3±6·2% (Table 2). In some sections there were 'files' of consecutively labelled cells at the base of the epidermis (Fig. 3) suggesting an active repair process in the damaged areas. The LI in the apparently uninvolved epidermis at the edge of the lesions in six patients was 12·3%, s.d.±2·9 (Table 2).

Fig. 1 diagrammatically shows the highest point reached by labelled cells in the biopsies that had been injected some days previously with tritiated thymidine. In some sections of the one and two

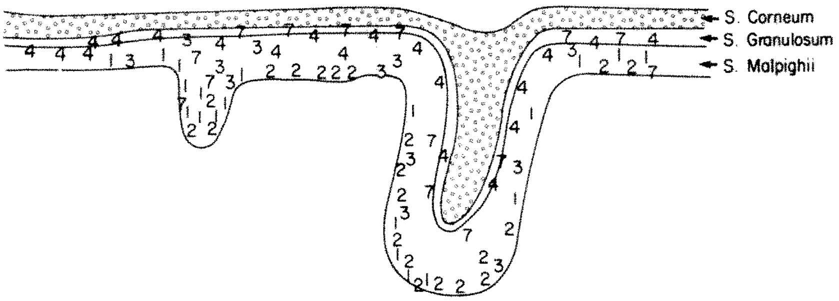

FIGURE 1. Diagram to show the highest point reached by labelled cells at the various time intervals (in days) between injection with tritiated thymidine (^{3}H Tdr) and biopsy of the injected site

Each number represents the number of days prior to which tritiated thymidine was injected and its site represents the highest point reached by labelled cells in one microscopic field (×45).

Nos. of patients injected with ^{3}H Tdr at various times prior to removal

Day	No.
1	1
2	4
3	1
4	2
5	0
6	0
7	1

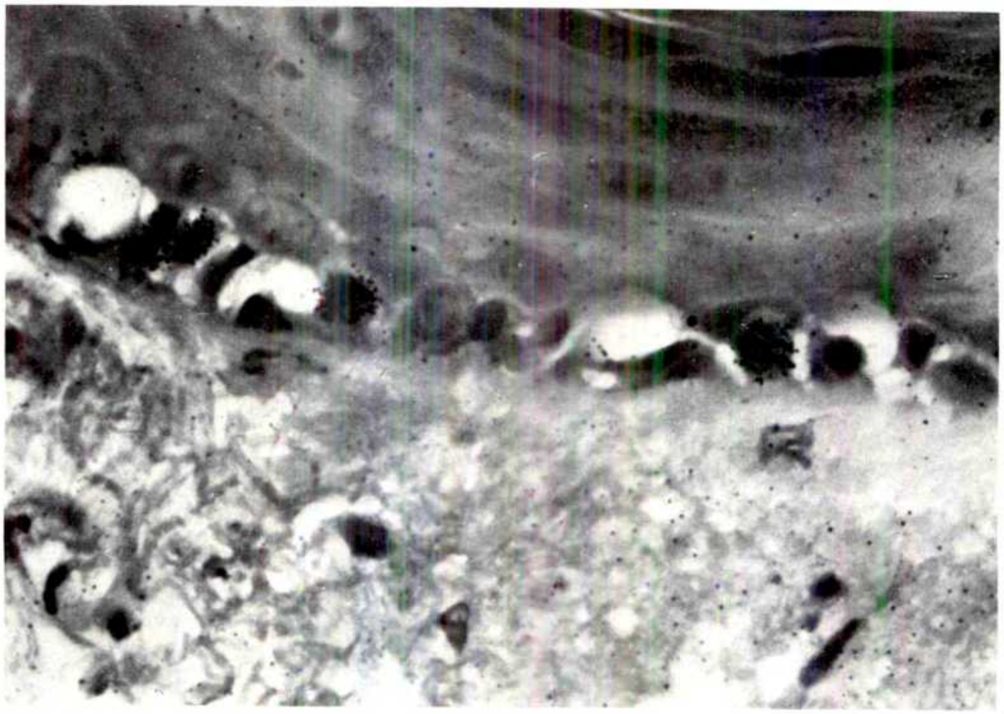

FIGURE 2. Photomicrograph of the dermo–epidermal junction showing labelled keratinocytes next to degenerate cells in the basal layer of the epidermis. The lesion was injected 1 h previously with tritiated thymidine (H & E, ×280).

day interval ARs, labelled cells had reached the mid epidermis. In some areas of the 4 day interval biopsies labelled cells had progressed as far as the upper Malpighian layer (Fig. 4) and even in a few places to the granular layer. In the 7 day specimens there were many areas where labelled cells had reached the subgranular area and in some there were labelled cells in the granular layer. In general the rate of transit was enhanced compared to normal epidermis and was certainly more rapid than that seen previously in a similar experiment in LP. There was not the same degree of irregularity of transit of labelled cells in the ARs from LE as had been seen earlier in LP (Marks *et al.*, 1973).

Immunofluorescence and histological findings

Of the 15 patients studied, direct immunofluorescence examination was performed in 13. The distribution and intensity of deposition of immunoglobulins in these 13 is set out in Table 3. Positive immunofluorescence findings were found in 11 of the patients. IgM was present as a linear band at the dermo–epidermal junction in all these patients often with strongly staining colloid bodies (Fig. 5). IgG was found in only 6 cases and was usually less prominent than IgM. Positive staining for fibrin was seen in 7 patients, in 4 of whom it was markedly prominent. In Case 2 it was strikingly heavy, ragged and similar to the pattern seen in lichen planus (Fig. 6).

The sections stained with PAS reagent demonstrated the following features:

(1) The PAS positive basement membrane was irregularly thickened and was thickest in the non-eroded areas of the plaque. (2) Where there was marked erosive change in the epidermis there often was apparent attentuation and fragmentation of the PAS positive band. (3) There was no consistent relationship between any morphological feature of the PAS positive subepidermal band and the numbers of cells in DNA synthesis in the autoradiographs.

TABLE 2. Labelling indices of lesional and surrounding 'uninvolved' skin

Patient	1 h LI of lesion (%)	1 h LI of 'uninvolved' perilesional skin (%)
1	12·2	8·5
2	19·4	—
3	13·2	9·0
4	16·5	—
5	19·5	—
6	15·8	—
7	23·5	—
8	23·0	14·5
9	30·4	14·0
10	29·0	—
11	28·0	15·0
12	25·0	13·0

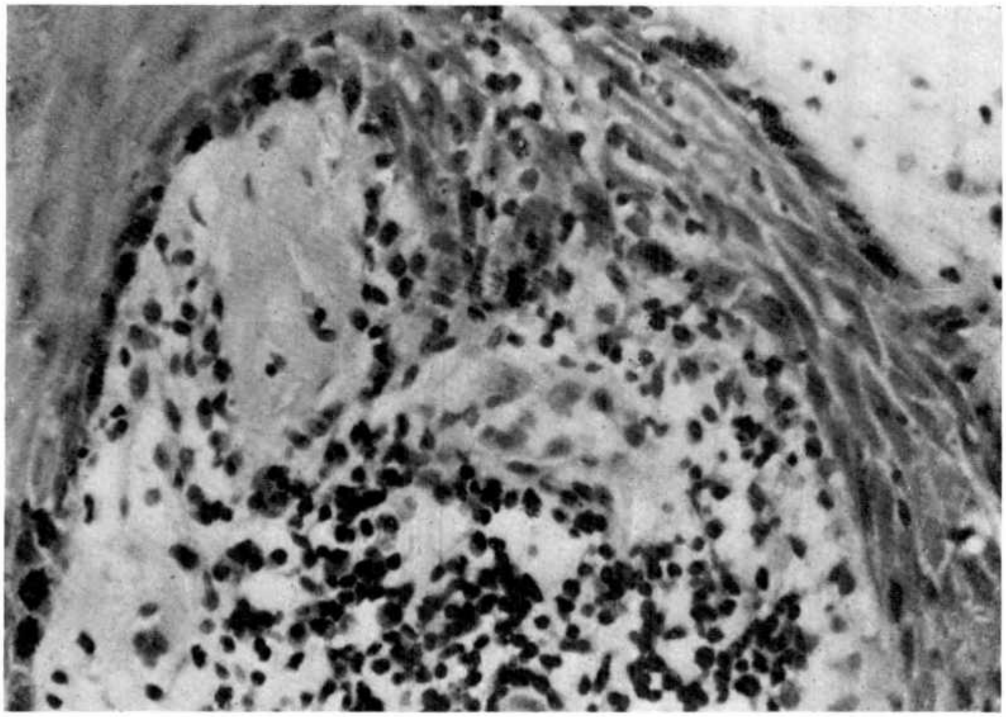

FIGURE 3. This photomicrograph shows 'files' of consecutively labelled basal keratinocytes 1 h following the injection of tritiated thymidine (H & E, × 150).

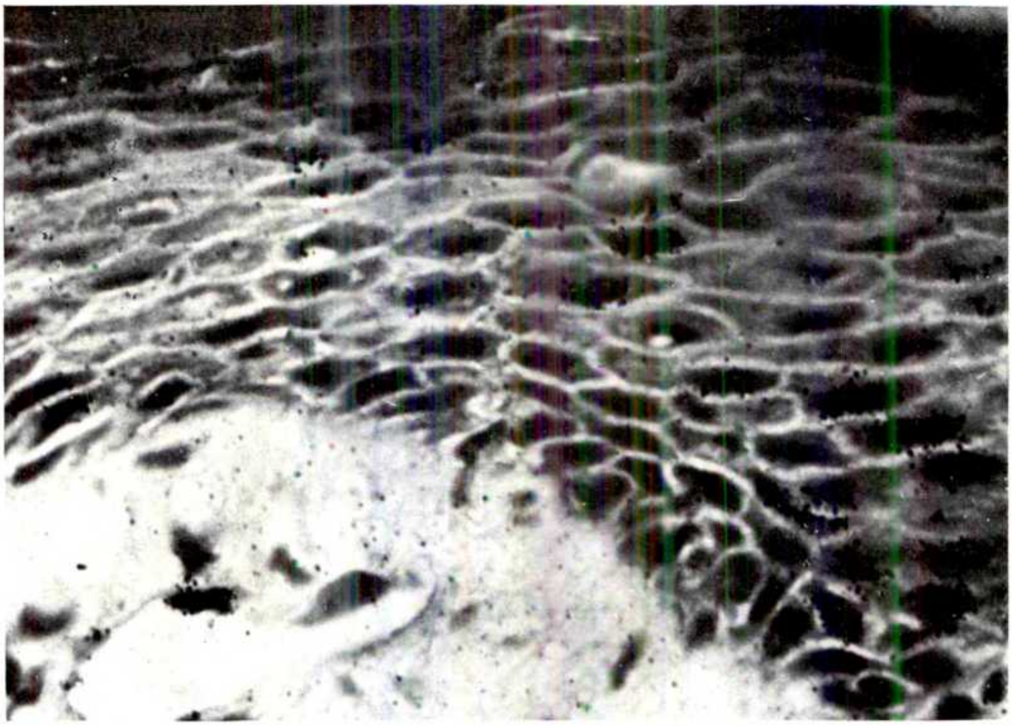

FIGURE 4. Photomicrograph of a lesion injected 4 days previously with tritiated thymidine. Labelled epidermal cells are seen throughout the Malpighian layer, reaching almost to the granular layer in places (H & E, × 150).

TABLE 3. Findings on direct immunofluorescence in 13 patients. The arbitrary scale ranged from absent (0) to very marked (+ + +)

	Arbitrary grading of intensity of deposit					
Patient no.	IgG	IgA	IgM	C^3	Fibrin	Comment
1	+	0	+ +	0	0	
2	+	0	+ + +	+	+ +	Cytoid bodies + with IgM Fibrin as seen in LP
3	+	0	+	0	+ +	
4	0	0	+ +	+	+ +	
5	0	0	0	0	0	
7	0	0	+ +	+ +	0	Cytoid bodies with IgM
9	0	0	+	0	+	Cytoid bodies with IgM
10	0	0	+ +	0	0	Cytoid bodies + + with IgM
11	+	0	+	+	+	
12	0	0	0	0	0	
13	0	ND	+	0	ND	Granular IgM
14	+	0	+	+	+	
15	+	0	+ +	+	+ +	

ND, not done.

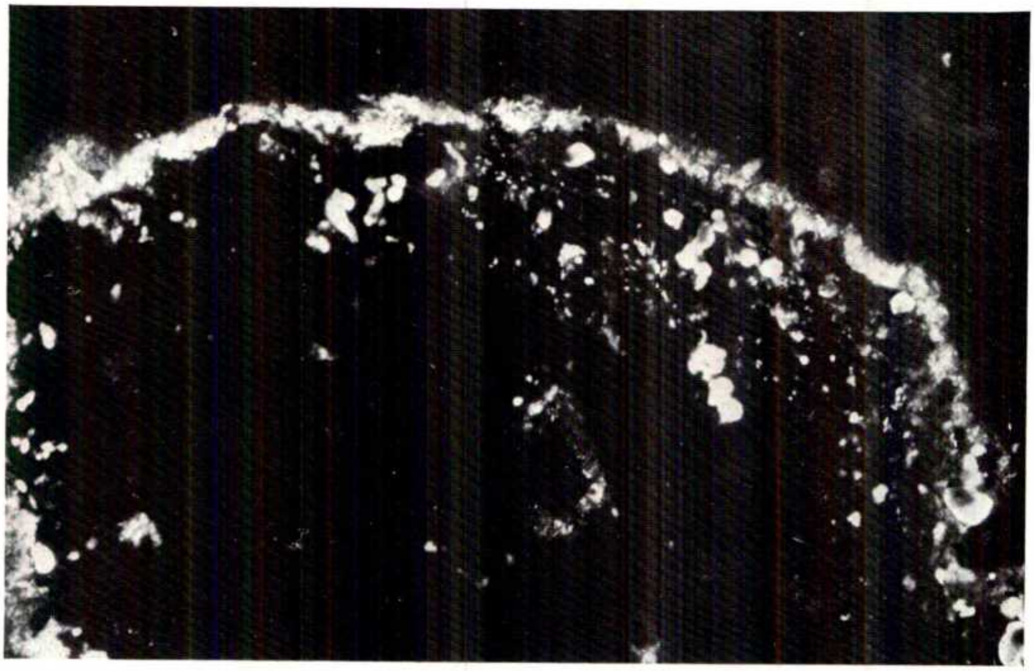

FIGURE 5. Immunofluorescence photomicrograph showing a thick band of IgM at the dermo–epidermal junction and IgM in colloid bodies (× 150).

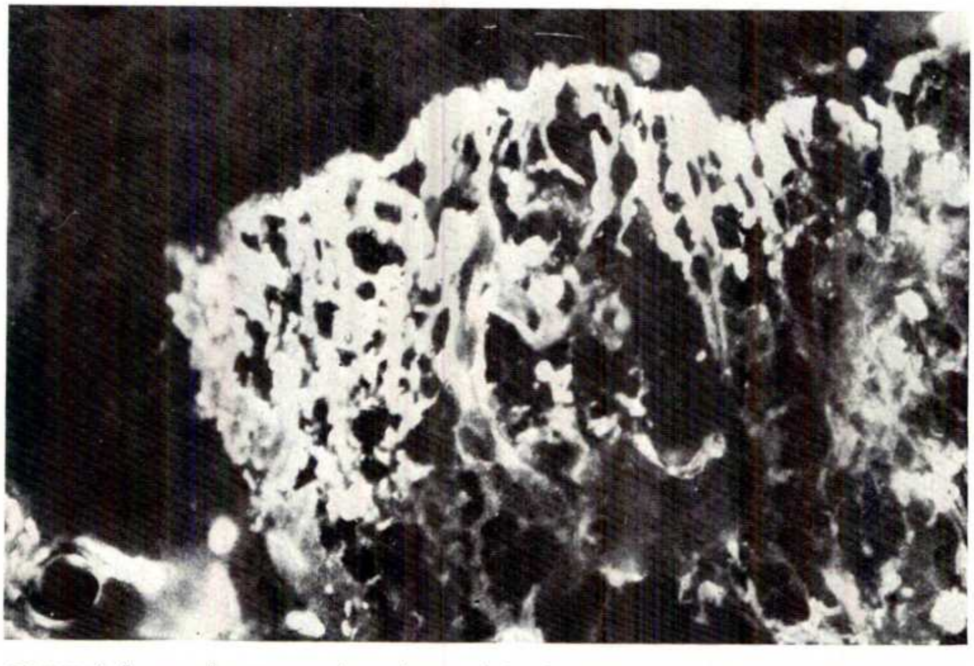

FIGURE 6. Immunofluorescence photomicrograph showing coarse, ragged deposition of fibrin in the upper dermis and at the dermo–epidermal junction. Fibrin is also present in colloid bodies and the appearances are typical of those seen in LP (× 150).

Close correlation between the distribution of IgM and the PAS positive band at the dermo–epidermal junction was observed in the 11 patients in whom there were positive immunofluorescence findings.

Epidermal cell migration studies

The degree of epidermal cell migration within the lesion and in the presence of obvious severe basal cell damage was often marked (Fig. 7) but was considerably less than that seen under similar circumstances in the lesion of LP (Presbury & Marks, 1974). Conversely there was more migration from the edge of the lesions of discoid LE than had been noted in the previous study of LP (Fig. 8).

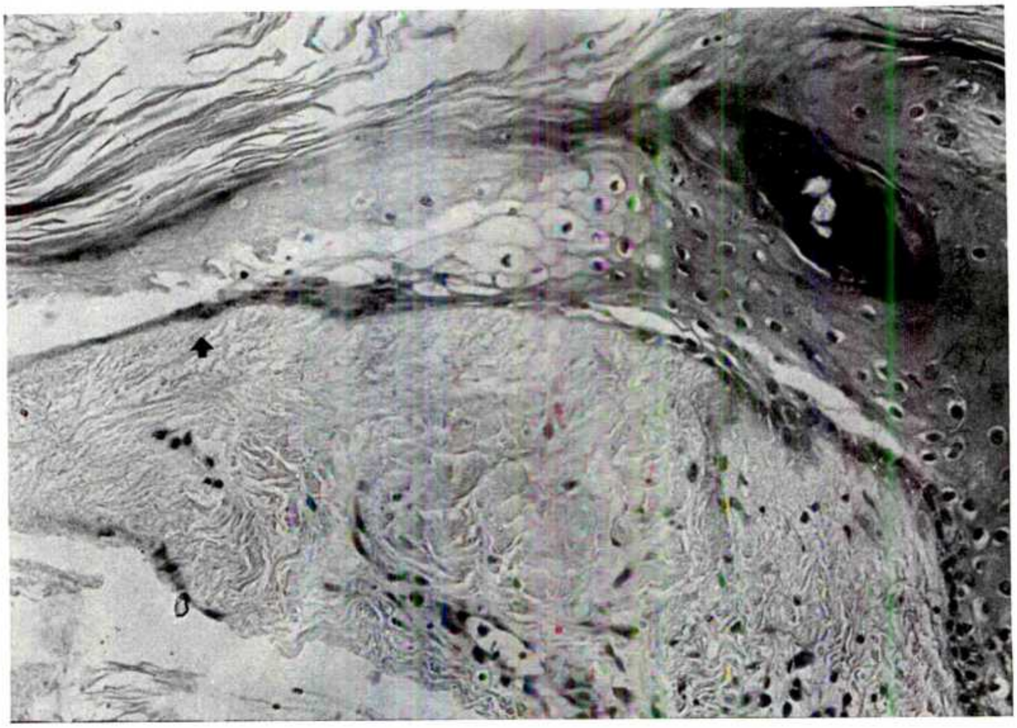

FIGURE 7. Photomicrograph of lesional skin incubated in Eagle's medium for 24 h. Epidermal cell death can be seen as can a 'file' of intralesional viable keratinocytes (↑) (H & E, × 150).

DISCUSSION

Discoid LE, in spite of the fact that it is far from rare, has received little attention from morphogenetic and pathogenetic points of view. Erosive damage to the basal compartment is, as in LP, a striking feature and this has been said to result in an epidermis with a slow 'turnover' of cells (Lever & Schaumburg-Lever, 1975).

The autoradiographs from biopsies taken 1 h after injection of tritiated thymidine demonstrate a very 'active' basal compartment of the epidermis, the labelling index of 21·3 ± 6·2 being even higher than that found in LP (Marks *et al.*, 1973). The presence of labelled epidermal cells interspersed with degenerate cells even in areas of severe basal erosion suggests a highly active repair mechanism. Moreover the stimulus for increased epidermopoiesis is operative even in the 'uninvolved' paralesional skin as once again seen in LP (Marks *et al.*, 1973). In a recent paper Brassine & Pierard (1976) found a similarly high 'flash' labelling index (29·3% ± 8·8%) in discoid LE lesions. Our autoradiographs prepared from biopsies removed 1–7 days after intracutaneous injection of tritiated

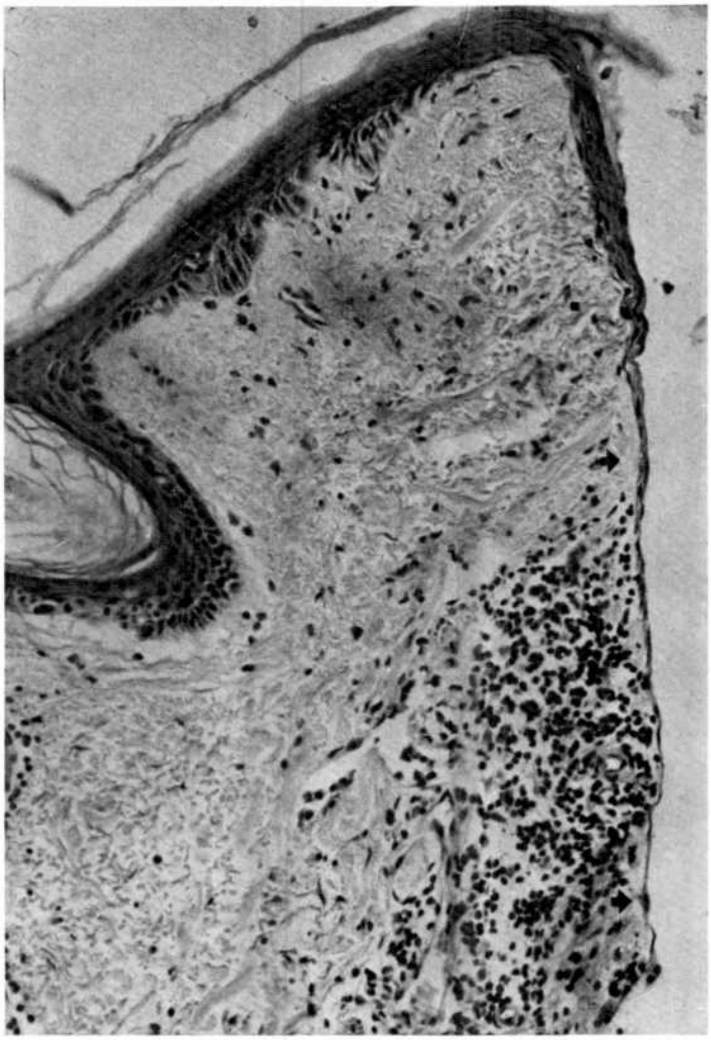

FIGURE 8. Photomicrograph of lesional skin incubated in Eagle's medium for 24 h, showing considerable epidermal migration (epiboly) at the edge of lesional skin (↑) (H & E, × 150).

thymidine showed a markedly decreased transit time for epidermal cells passing through the Malpighian layer. As in LP the actual rate showed variation between lesions and even within individual lesions. This could be explained by differing rates of mitosis in the basal compartment. However, we believe that a more likely explanation is that there is some variability in the rate of cell destruction after mitosis within the lesions. Further support for this concept comes from the surprising uniformity of labelling found in the 1 h ARs.

The large number of cells entering the mitotic process is at least in part explained by the recruitment of basal cells from adnexae (especially sweat ducts) and surrounding less eroded epidermis with some lateral movement of these cells. Support for this latter mechanism comes from the *in vitro* studies using small pieces from discoid LE lesions. Both intralesional migration and encircling migration (epiboly) were seen. Interestingly, epiboly was more pronounced than was seen in LP whereas intralesional migration was significantly less (Marks *et al.*, 1973). How might this decreased intralesional migration be explained in discoid LE? It might be a blocking effect from the immunoprotein and fibrin present at the dermo–epidermal junction and demonstrated by the immunofluorescence and PAS studies. In this context it is possibly pertinent that the larger IgM rather than IgG was the predominant immunoglobulin at the dermo–epidermal junction. In systemic lupus erythematosus IgM has also been reported as being the most prominent immunoglobulin (Ten Have-Opbroek, 1966; Pohle & Tuffanelli, 1968; Jordon, Schroeter & Winkelmann, 1975) although similar claims have been made for IgG by other workers (Cormane *et al.*, 1966; Tan & Kunkel 1966; Burnham and Fine 1969).

We suggest that the epidermal reparative response to erosive damage to the basal compartment is very active in discoid LE. The 'blocking' effect of the abnormal deposits at the dermo–epidermal junction could at least partially explain the usually different but occasionally similar epidermal architecture in discoid LE in comparison with LP. The striking intralesional epidermal cell movement in LP may not be seen in discoid LE because of these protein deposits and this could well influence the resulting reparative process. A further mechanism that may well be operative in determining the difference in overall morphology of the two diseases is the probable difference in the rate and severity of basal cell damage.

This study was not directly concerned with the immunopathogenesis of discoid LE but we were impressed by the number of eroded areas that were not infiltrated with lymphocytes. This might suggest that humoral damage initiates degenerative changes in the basal compartment and that the arrival of lymphocytes subsequently is secondary to this.

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Case Reports

Generalized eczéma craquelé as a presenting feature of lymphoma

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SUMMARY

Two cases of lymphoma are reported—in both, extensive eczéma craquelé resistant to treatment was a presenting feature.

Although eczéma craquelé is a common condition, there are very little data on its incidence, pathogenesis or treatment. Recently Warin (1973) discussed the history of the disorder and described three patients with advanced myxoedema who presented in this way.

Whilst it is well recognized that acquired ichthyosis may be associated with Hodgkin's disease (Stevanovic, 1960), the combination of lymphoma with eczéma craquelé has not been reported before.

CASE REPORTS

Case 1

A 69-year-old man presented with a month's history of anorexia, lassitude and a generalized itchy, dry and scaly skin. The rash had not responded to treatment with oil baths and was getting rapidly more extensive. There was no previous history of skin disease except for 'industrial dermatitis' many years previously. However, he gave a 12-year history of coeliac disease treated with a gluten-free diet to which, he stated, he adhered strictly.

On examination he had almost generalized classical eczéma craquelé involving shins, thighs, shoulders and back (Figs 1 & 2). Discrete lymphadenopathy was detected in the right axilla, but there was no enlargement of the liver or spleen.

Investigation revealed Hb 12·4 G/dl with a total white count of $9{\cdot}6 \times 10^9$/l and a persisting eosinophilia of 25–35%. Renal and liver function tests were initially normal. There was evidence of continuing malabsorption in that faecal fat excretion was raised; and only 13% of an oral xylose dose was excreted by 5 h. There was, however, no evidence of essential fatty acid malabsorption. Chest X-ray showed a left basal effusion with enlarged superior mediastinal glands.

The patient was transferred to the Thoracic Medical Department (Dr Gordon Edwards). Sputum cytology and pleural biopsy were negative, but histological examination of a supraclavicular node revealed lymphoma, probably of Hodgkin's type. During his admission the patient received conventional treatment for his eczema. Only frequent and liberal applications of betamethasone valerate

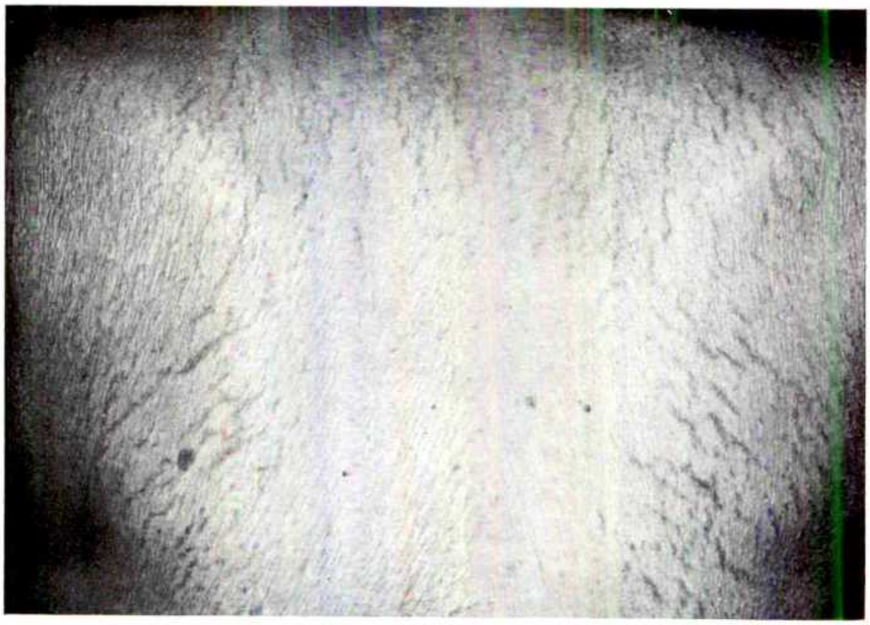

FIGURE 1. Eczéma craquelé on the back of Case 1.

ointment 25% in emulsifying ointment were of any help, but even so, improvement was only partial and transient. Sunflower oil applied topically had no beneficial effect on the skin.

After a period of chemotherapy the patient died. Post mortem examination confirmed the diagnosis of Hodgkin's disease.

Case 2

A 65-year-old man presented with a 3-week history of anorexia, malaise and a generalized dry, scaly,

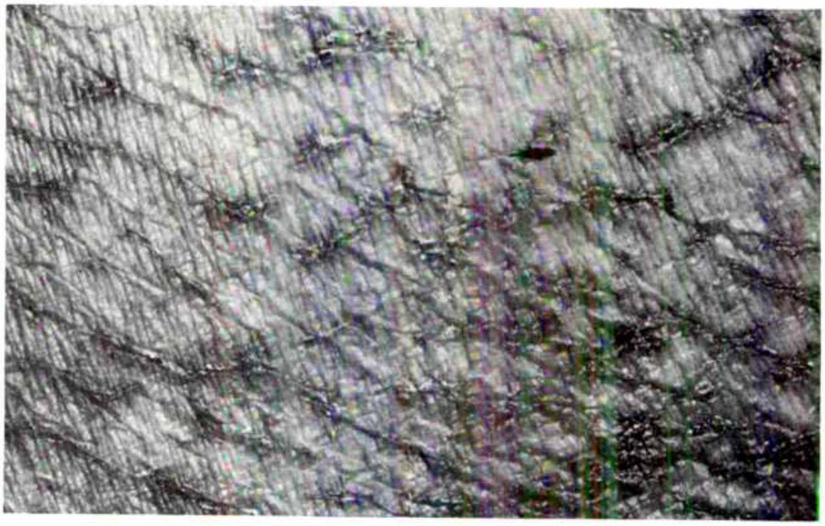

FIGURE 2. Eczéma craquelé in Case 1 demonstrating purpuric fissures.

itchy rash. There was no previous history of skin disease, or any other medical problems except for a mild depressive illness.

On examination he was ill and febrile with marked eczéma craquelé over thighs, shins and abdomen. Small, firm lymph nodes were present in both supraclavicular fossae. There was no enlargement of liver or spleen.

Investigation revealed a persisting anaemia with an ESR of 135 mm/h. Hepatic enzymes were elevated (alkaline phosphatase 23·1; SGOT 24). There was biochemical evidence of hypothyroidism (Total thyroxine 50 nmol/l; free thyroxine index 0·95) but the patient was clinically euthyroid.

Right axillary lymph node biopsy showed Hodgkin's disease (Dr C.K. Anderson). The patient was referred for chemotherapy, but his condition rapidly deteriorated. Autopsy confirmed widespread Hodgkin's disease of the lymphocyte depleted type.

During the last few weeks of his life this patient's dermatosis persisted despite treatment with dilute topical corticosteroid ointments.

DISCUSSION

The irregular, reticulate, dry fissured appearance of eczéma craquelé is a common sight localized mainly to the shins of elderly hospital in-patients. The degreasing effect on the skin of regular and methodical washing, the dry, starched sheets and low environmental humidity are probably all, in part, responsible.

In a personal study of all admissions to an orthopaedic unit during a 6-month period the authors found that 20% of patients ultimately developed a pattern of pronounced scaling and fissuring of the lower legs and feet. In those confined to bed for more than 2 weeks with skeletal injuries the number developing these changes was far higher, nearly 70%. Only three of the 130 patients followed developed frank eczéma craquelé, localized to shins and thighs, all within the first month following admission. These, and all other patients affected and seen by the authors during the preceding year were over 50 years of age. This may, however, reflect only the age distribution of the hospital in-patient population. None of these patients was clinically hypothyroid and all responded promptly to treatment with a corticosteroid ointment diluted in emulsifying ointment.

The skin changes seen in the two patients reported here were far more extensive than is usually seen in the common form of eczéma craquelé described above. Moreover, the patients differed in their refractory response to treatment which is usually effective.

The pathogenesis of the eruption remains an enigma. Both our patients were clinically euthyroid although the second case had some biochemical evidence of hypothyroidism.

Chronic malabsorption has recently been associated with essential fatty acid deficiency (E.F.A.) and a scaly dermatitis (Prottey, Hartop & Press, 1975). There was no evidence of E.F.A. malabsorption in the first case, or response to topical sunflower oil, rich in linoleic acid.

Murray & Forsey (1975) described a patient with a marked reduction in sweating and eczéma craquelé, in an area of traumatic denervation. There was no obvious clinical evidence of peripheral neuropathy in our patients but we did not attempt to evaluate sweat gland function.

In summary, the rapid onset of extensive eczéma craquelé, coupled with resistance to treatment, should alert the clinician to the possibility of an underlying lymphoma. This form of eczéma craquelé should not be confused with the localized dermatosis commonly seen in elderly and often bedridden hospital in-patients.

ACKNOWLEDGMENTS

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Acquired ichthyosis: multiple causes for an acquired generalized disturbance in desquamation

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SUMMARY

Four patients with acquired ichthyosis are described and the results of histological, histometric, autoradiographic and enzyme histochemical studies reported. Three of the patients were found to have in addition the following underlying diseases—malnutrition, reticulum cell sarcoma and pan-hypopituitarism. The fourth patient did not have any systemic disorder and the provoking cause of his ichthyotic condition remains obscure. Clinically these patients demonstrated the skin changes of autosomal dominant ichthyosis although their condition tended to fluctuate in severity. The various studies performed indicated that the histological and metabolic changes tended to reflect the clinical state of the patients and that these parameters did not differ significantly from those observed in autosomal dominant ichthyosis.

Most cases of ichthyoses are genetically determined and include the common autosomal dominant and sex linked ichthyoses and rarer autosomal recessive diseases such as lamellar ichthyosis and non-bullous ichthyosiform erythroderma. These conditions usually become apparent in the first few months of life. However, a similar clinical state occasionally arises during adult life, and is clinically and histologically indistinguishable from the genetically determined ichthyoses. Such 'acquired ichthyosis' is usually ascribed to an underlying malignancy, particularly the reticuloses and especially Hodgkin's disease (Sneddon, 1955; English *et al.*, 1963; Majekodunmi & Femi-Pearse, 1974; Flint, Flam & Soter, 1975). The characteristic dryness and scaliness usually appear some time after other manifestations of the malignant process. Less often these changes coincide with or even precede the malignancy by as much as 7 years (Stevanovic, 1960). Treatment of the underlying malignancy often leads to an improvement in the ichthyosis (Sneddon, 1970.) Acquired ichthyosis is not only seen as a manifestation of a neoplastic disorder. It has also been reported in essential fatty acid deficiency following intestinal resection (Press *et al.*, 1974) and in leprosy (Schultz, 1965). Other reported causes include renal disease with an associated defect in proline metabolism (Goyer *et al.*, 1968) and after administration of drugs which block the biosynthesis of cholesterol (Simpson, Blair & Cranswick,

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1964). In this paper we report four cases of acquired ichthyosis and the results of investigations into the structure and metabolic activity of the epidermis in these patients.

PATIENTS AND METHODS

The clinical histories and results of examination are as follows.

Case 1. A 60-year-old man with a long history of alcoholism and poor food intake. The onset of dryness and scaliness was recent with no previous history of skin problems. On examination, he was found to be thin and malnourished with widespread dryness and fine scaling, particularly on the limbs. He was admitted to hospital and rapidly improved on a normal diet with included vitamin and folic acid supplements. He was discharged but failed to attend for follow-up.

Case 2. A 57-year-old male with several months history of lymphadenopathy in the left axilla which discharged on occasions. This failed to respond to antibiotic treatment. An area of erythema and scaling around this swelling was apparent at this time. A biopsy was performed and subsequent histological investigation enabled a diagnosis of reticulum cell sarcoma to be made. Eventually, lymphadenopathy was evident in both axillae and groin together with a generalized ichthyosis. On examination, the skin was noted to be very scaly and slightly erythematous over both trunk and limbs. The ichthyosis improved with treatment of the lymphoma by chemotherapy but the patient later relapsed and died at home. The condition of his skin at the time of death is unknown.

Case 3. A 15-year-old boy with a 6-month history of dryness and scaliness which apparently started on the legs and soon spread to the rest of the body. Recently this had become more severe but he was otherwise well. The ichthyosis was most severe on the arms and legs with large brownish shield-like scales. The scalp was also involved. Extensive investigations failed to find an underlying cause and the ichthyosis is still evident though with emollients less severe.

Case 4. A 61-year-old man with a long history of dry, scaly skin and loss of body hair over approximately 14 years prior to a diagnosis of pan-hypopituitarism. Periods of severe ichthyosis were noted more recently, possibly related to lapses in treatment. On examination, large, whitish, polygonal scales were noted on trunk and limbs. After a recent reassessment of replacement therapy, the ichthyosis improved together with more growth of facial and body hair. However, his skin still remains basically dry.

The results of some relevant laboratory investigations on these patients are summarized in Table 1.

Epidermal structure and metabolic activity

All patients had skin biopsies which were examined histologically and by enzyme histochemical methods. As a measure of epidermal architecture the mean epidermal thickness (MET) and the rete pattern, as defined by the ratio of the length of the basal layer to the length of the granular layer (B:G ratio), were determined (Holt & Marks, 1976). The enzyme histochemical tests were performed as described by Chayen, Bitensky & Butcher (1973), on cryostat sections from skin specimens that had been quenched in hexane at −70°C.

Epidermal growth

To obtain an expression of the generative activity of the epidermis, skin fragments from three of the patients were incubated in Eagle's minimal essential medium containing tritiated thymidine. In two

TABLE 1. Relevant laboratory investigations

Case no.	Haematological	Serum iron and total iron binding capacity (TIBC)	Serum urea and electrolytes	Liver function test	Serum lipids
1	Hb = 10·2 g/dl WBC = 10·1 × 10^9/l Bone marrow showed megaloblastic changes associated with folate deficiency	Serum iron = 10·2 µmol/l TIBC = 24·4 µmol/l	Urea = 1·0 mmol/l Electrolytes normal	Serum albumin = 26 g/l SGPT = 62 I.U./l otherwise normal	—
2	Hb = 12·0 g/dl WBC = 20·8 × 10^9/l	—	Urea = 1 5 mmol/l Electrolytes normal	Serum albumin = 31 g/l otherwise normal	—
3	Hb = 12·9 g/dl WBC = 9·9 × 10^9/l	Serum iron = 6·5 µmol/l TIBC = 60·4 µmol/l	Urea and electrolytes normal	Normal	Triglycerides and cholesterol normal. Total fatty acid analysis indicated < 0·5% 5,8,11–eicosatrenoic acid
4	Hb = 10·3 g/dl WBC = 12·6 × 10^9/l	Serum Iron = 16 0 µmol/l TIBC = 65 µmol/l	Urea and electrolytes normal	Normal	Triglycerides and cholesterol normal. Total fatty acid analysis indicated < 0·5% 5,8,11–eicosatrenoic acid

TABLE 2. Histology, epidermal measurements, enzyme histochemistry and labelling indices

		Enzyme Histochemistry							
Case no.	Histology	Non specific esterase	Acid phosphatase	Glucose-6 phosphate dehydrogenase	Lactate dehydrogenase	Succinic dehydrogenase	B/G ratio	M.E.T. (nos of cells)	L.I. (%)
1	Slight acanthosis but decrease in thickness of granular cell layer. Normal rete pattern	A slightly thinner than normal band Normal intensity	Normal	Normal accentuation in upper epidermis	Normal uniform distribution	Strong normal distribution	1·06	4·8	3·0
2	Some irregularity of dermo–epidermal junction. Marked hyperkeratosis and absence of granular cell layer	A slightly thinner than normal band. Normal intensity	Normal	Normal accentuation in upper epidermis	Normal uniform distribution	Normal uniform distribution	—	—	—
3	Irregular acanthosis with 'church spire' appearance of variable degree. Marked hyperkeratosis. Unusual vacuolated appearance of upper epidermis. Granular cells are present but seem deficient in numbers and granules	Broadened band up to a third of the thickness of the epidermis	Normal	Normal accentuation in upper epidermis	Normal uniform distribution	Some decrease in upper epidermis	1·45	6·9	8·4
4	Thinning of epidermis. Relative hyperkeratosis. Absent granular cell layer. Flattening of dermo–epidermal junction	Narrowed band	Normal	Normal accentuation in upper epidermis	Normal uniform distribution	Normal uniform distribution	0·99	3·8	5·8

instances (Cases 1 and 4) the skin samples were obtained from the thigh with a Castroviejo keratotome set at 0·4 mm. The specimens were incubated in media containing tritiated thymidine of specific activity 2 Ci/mmol and at a concentration of 1 μCi/ml for 4 h. At the end of the incubation the skin was fixed in 10% buffered formalin and processed histologically and autoradiographically (Joftes & Warren, 1955). In Case 3 a wedge biopsy from the lower back was cut into thin slices approximately 1 mm thick and incubated for 2·5 h in media containing tritiated thymidine of specific activity 18 Ci/mmole at a concentration of 2·5 μCi/ml. These slices were then processed histologically and autoradiographically as above. The labelling indices (L.I.) were calculated from the autoradiographs as the percentage of labelled basal and suprabasal epidermal cells per 1000 basal cells.

RESULTS

The results of histological, enzyme histochemical, histometric and autoradiographic studies are set out in Table 2. Cases 2 and 3 are illustrated in Figs 1 and 2 respectively. Photomicrographs of haematoxylin and eosin sections from biopsies of Cases 3 and 4 are presented in Figs 3 and 4 respectively. There was hyperkeratosis and irregular acanthosis in all four cases as well as diminuation in granular cell layer thickness. However, there were subtle variations from patient to patient as is clear from

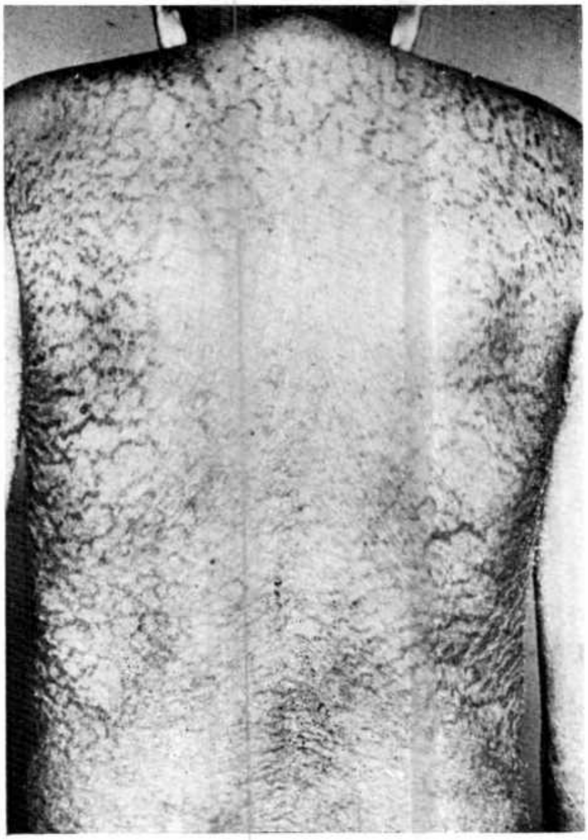

FIGURE 1. Case 2.

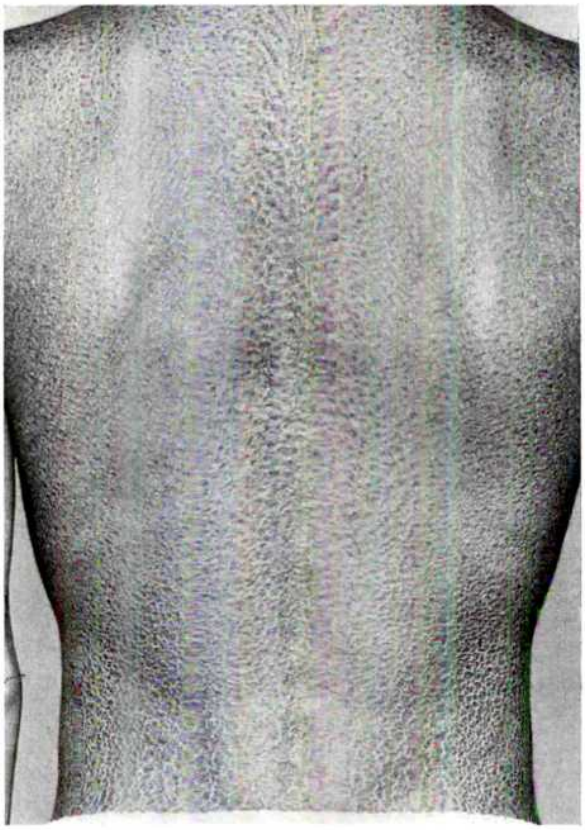

FIGURE 2. Case 3.

Table 2. The labelling indices show a normal or slightly reduced proportion of labelled cells when compared to the normal interfollicular epidermis (Shahrad & Marks, 1976). This accords with a similar observation made in autosomal dominant ichthyosis (Frost, 1973). The enzyme histochemical tests demonstrated essentially normal patterns although the non-specific esterase reaction in Case 3 was broadened and decreased in Cases 1, 2 and 4.

DISCUSSION

The four patients that we have described all developed an ichthyosis-like condition during adult life. The fact that four such patients have been seen within 1 year is, perhaps, a reflection of our interest in disorders of keratinization and desquamation and probably not due to any special environmental considerations. We cannot say whether the ichthyotic condition in our patients had the same root cause but they certainly had overall clinical and pathological similarities. One unexpected common denominator was the slight anaemia in the four patients (Table 1). It is of interest in this respect that iron deficiency has been documented as a cause of pruritus (Vickers, 1973) and oral cornification (Jacobs, 1959). Purists may cavil at our use of the term ichthyosis to describe one or another of our cases primarily because of the lack of any universally accepted definition of this disorder. The ideal would be an accurate delineation in terms of the underlying biochemical fault. This is possible in Refsum's disease for example and to a limited extent in essential fatty acid deficiency. A working

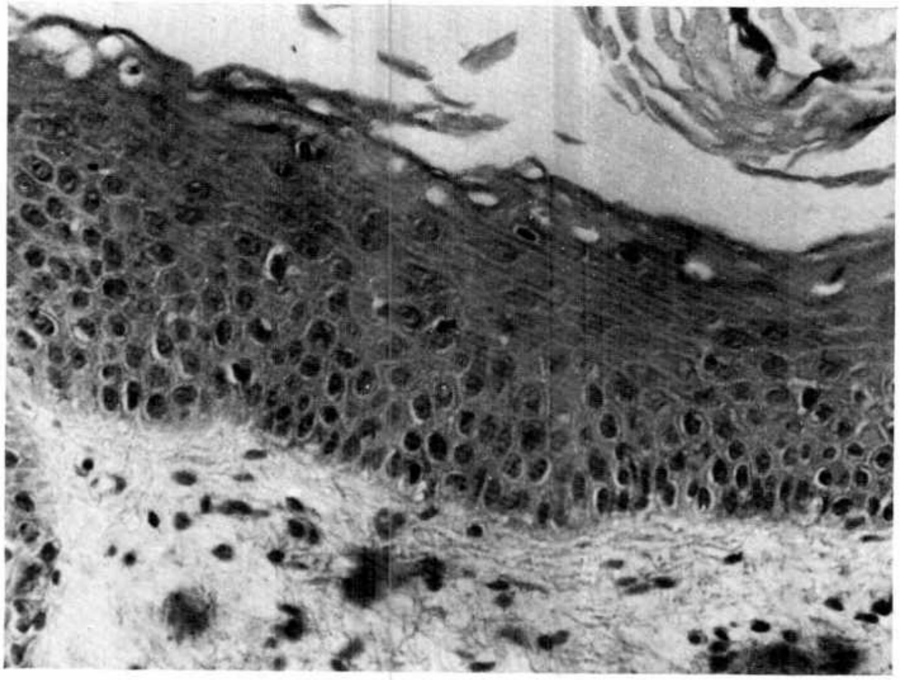

FIGURE 3. Section of skin from Case 3 (H & E, × 400).

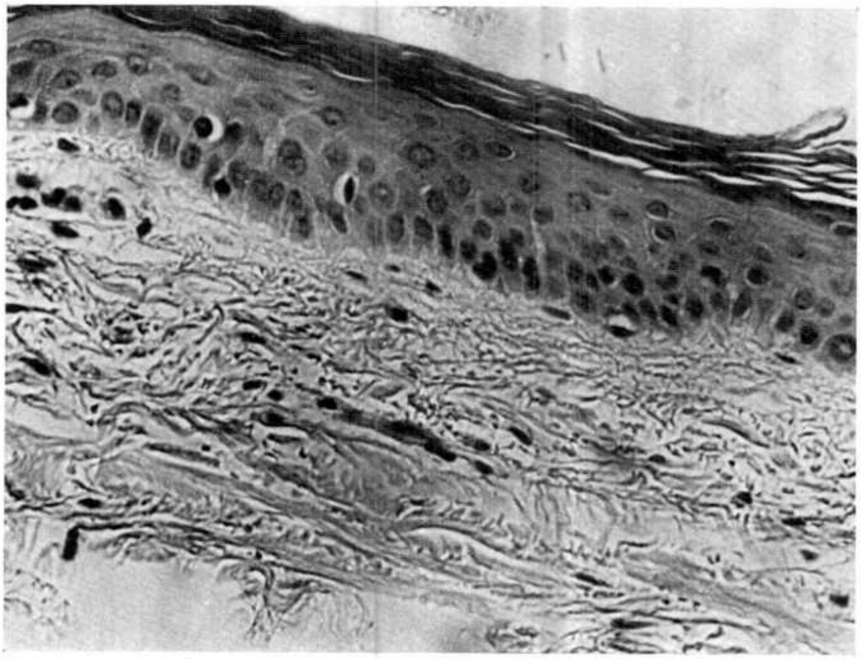

FIGURE 4. Section of skin from Case 4 (H & E, × 400).

definition might be, a generalized abnormality of the skin surface characterized by dryness and roughness in which large clumps of desquamating corneocytes are shed in the absence of signs of inflammation. We believe that this definition is more likely to group together disorders which have a common pathogenesis and throw some light on the pathophysiological mechanisms involved.

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Darier's disease: a kindred with a large number of cases

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SUMMARY

Over 70 cases of keratosis follicularis (Darier's disease) were noted in a study of a single kindred consisting of more than 200 individuals. Data were obtained through questionnaires, interviews and physical examinations. Results strongly support an autosomal dominant mode of inheritance since the ratio of affected to unaffected members was 1:1 and there were nearly equal numbers of males and females with this condition. Large sibships were common in this kindred, which extended over seven generations, and most of the affected individuals were located in the southwestern New York–northwestern Pennsylvania region of the United States.

Although Darier's disease is a well-defined clinical entity (Darier, 1889), the pathogenesis is unknown. The incidence is familial and the pattern of inheritance appears consistent with that of an autosomal dominant disorder with manifestations usually appearing in adolescence (Ebling & Rook, 1972). The prevalence of this rare trait in the population of Denmark was found to be only 1 in 100,000 by Svendsen & Albrechtsen (1959). While reports of isolated cases of Darier's disease have been numerous, familial case reports are less common. Most contained accounts of just a few affected individuals and the kindreds seldom extended beyond one or two generations. Consequently, the ratio of affected to unaffected individuals is usually far from the expected 1:1 ratio and Cockayne (1933) has suggested that infertility, frigidity or difficulty in finding a marriage partner are responsible.

The treatment of many cases of this disease in the immediate area of southwestern New York prompted a search of the family histories of the patients. This led to the discovery of what appears to be the largest kindred ever reported for this disease.

METHOD

Relatives of known cases were contacted through questionnaires. These requested information on the individual's age, sex, and physical stature; presence or absence of the disorder, circumstances surrounding its onset and any medical treatment received; and names and ages of immediate relatives (affected and unaffected). Answers and additional details were further elaborated upon or confirmed

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by correspondence, telephone or by personal interviews with various members of this kindred in their homes or the physician's office.

Darier's disease was diagnosed clinically by one of the authors (A.L.B.) or by other dermatologists certified by the American Board of Dermatology (see Acknowledgment). Diagnoses were based on the following criteria: typical greasy, yellow to brown crusted papular lesions found mainly in the seborrhoeic areas of the head, neck and trunk. These lesions were malodorous in flexural areas. Most of the patients exhibited discrete, papular verrucose lesions over the dorsa of the hands, and punctate keratoses of the palms and soles so often reported with this disease (Ebling & Rook, 1972).

Diagnosis was confirmed by histological examination of biopsied skin, particularly if any doubt existed about the clinical diagnosis. Histology showed dyskeratosis resulting in corps ronds and grains. Suprabasalar lacunae, papillomatosis, acanthosis and hyperkeratosis were features. The dermis showed a chronic inflammatory infiltrate. Darier's disease was confirmed clinically and/or histologically in 26 individuals. In one case, Darier's disease can be inferred directly from genetic evidence (Fig. 1, V 8).

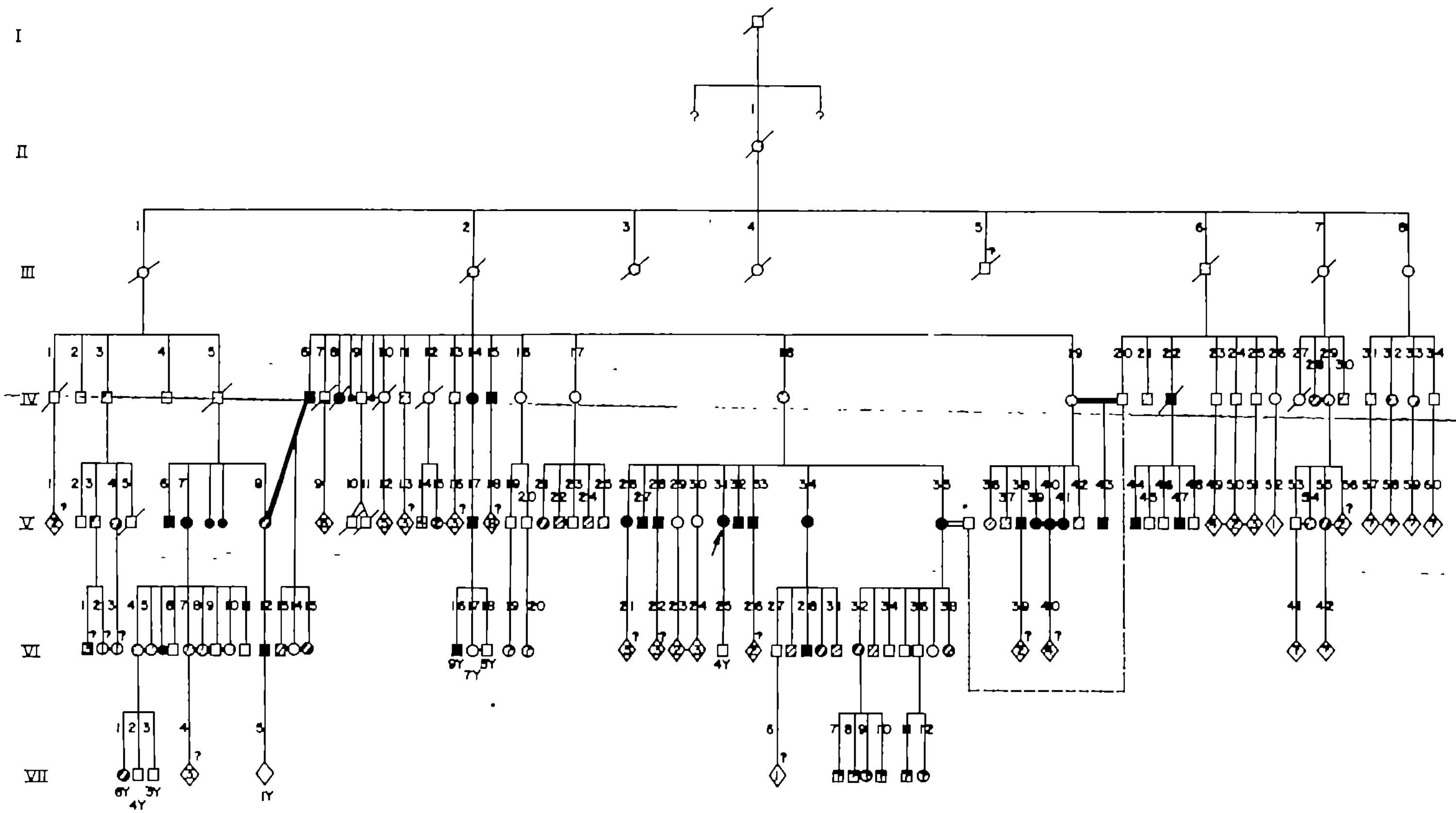
Many other cases were discovered and some were visited but were not examined clinically for confirmation and thus the diagnosis of Darier's disease was considered presumptive. An individual was presumed to have the disease when reported to be affected by at least two relatives. There appeared to be little confusion in the kindred concerning the distinction between affected and 'clear' members. In 61 of 64 cases (95·1%), members of the kindred correctly identified the confirmed status of other members of the kindred not in their immediate family. For these reasons we are confident that individuals of this lineage presumed to have the disease are actually affected.

RESULTS

The results of this study are summarized in the pedigree shown in Fig. 1. Of 202 individuals spanning a period of seven generations, 26 (16 males and 10 females) confirmed and 48 (19 males and 29 females) presumptive diagnoses of Darier's disease were uncovered. A ratio of affected to unaffected individuals of nearly 1:1 (74:70) was obtained. Excluded from this ratio were 52 individuals of unknown clinical status, many of whom (35) were children of affected or presumed affected persons, as well as children (6) under 10 years of age. The latter were omitted since onset of symptoms generally occurred in adolescence. However, two children under 10 years of age with confirmed Darier's disease (VI–16 and VII–1, Fig. 1) were included among the affected, introducing a slight bias of ascertainment.

The data support an autosomal dominant mode of inheritance as every affected individual had (at least) one affected parent. While there were slightly more females (39) than males (35) with this condition when both confirmed and presumptive cases were considered, the difference was not significant ($P > 0{\cdot}6$) and again approximated a 1:1 ratio.

Even though most of those in the first three generations are now deceased, the disease could be traced back to a male Irish immigrant (I–1, Fig. 1) whom decendants implicated as the 'original' source. None of those still living, however, could trace the horizontal lineage in generation II (Fig. 1). Parental consanguinity was a feature of two marriages in this kindred. A marriage between cousins (male, IV-6 and female, V-8; Fig. 1), both of whom had Darier's disease, produced two affected (VI-13, 15) and one unaffected (VI-14) offspring. The latter was a late adolescent, however, and could conceivably develop the symptoms later. In the second case, a first cousin mating (IV-19 and IV-20, Fig. 1) produced one affected offspring (V-43). The unaffected male parent (IV-20) subsequently fathered seven offspring (three of whom were presumed affected) with the niece (V-35) of IV-19. Both females (IV-19 and V-35, Fig. 1) had Darier's disease.



■ Male, confirmed Darier's syndrome; ○ Female, unaffected; ∅ Deceased; ⊘ or ▨ Presumptive, not confirmed, ? Sex not specified, status unknown, ⊞? 4 male offspring, status unknown; □ 5Y Age (years) of young (under 10) children

FIGURE 1. Pedigree of a kindred with Darier's disease. Arrow indicates index case.

Affected individuals in this kindred have dispersed primarily throughout the Allegheny mountain region of the U.S. in southwestern New York and northwestern Pennsylvania. A relatively high fertility existed among those with Darier's disease examined in this study, the number of living offspring in the seven largest families being 14, 10, 9, 8, 8, 7 and 7, respectively. While mean numbers of offspring (living) among adults with confirmed and presumptive Darier's disease (3·0 ± 3·5) exceeded the mean number of offspring produced by 'clear' relatives (1·8 ± 1·9), the differences were not significant. However, when the mean number of offspring of matings having at least one parent with Darier's disease (4·4 ± 2·9) was compared with that (2·8 ± 1·7) of matings between unaffected parents, the difference was significant ($P < 0·05$, Mann–Whitney U test).

DISCUSSION

A study of Darier's disease among 202 related individuals has revealed the largest number (74) of cases ever reported. Hambrick & Leedham (1950) reported the largest number of cases of Darier's disease among relatives to that date, with 10 affected members extending over a period of four generations. Subsequently, Svendsen & Albrechtsen (1959) presented five kinships in their study of this disease in Denmark. The largest of these contained 13 cases in a five-generation span of 132 individuals. The kindred described in the present study had twice the number of cases with confirmed diagnoses and, if cases with a presumptive diagnosis are included, had over five times the number of affected individuals previously reported to exist in a single kindred. The total even exceeded the number of cases reported in the Denmark study. It is conceivable than an even greater number of cases would have been uncovered were it possible to trace the horizontal lineage of generation II in this pedigree (Fig. 1).

Data presented here confirm the autosomal dominant mode of inheritance of this trait. The ratio of affected to unaffected members was 74:70 after adjusting the total for those of unknown status and for children under 10 years of age. Children under 10 years of age as well as the first generation were also excluded from consideration in the Denmark study, where the ratio of affected to unaffected was 26:34. Here as in the Denmark study, the disorder was equally distributed between the sexes.

Of 51 cases of Darier's disease spread throughout twenty-two families in the Denmark study, 14 were deceased, leaving 37 cases in a population of 4,454,000. The prevalence of this disorder was therefore about 1 in 120,000 and Svendsen & Albrechtsen (1959) suggested that an increase in the incidence of this disorder might point to the existence of mutation-provoking factors. Ebling & Rook (1972) state that many cases arise as new mutations. While isolated cases may be due to mutation, an increase in the frequency of this phenotype could be revealed through a more complete ascertainment of the disease among relatives as in such studies as are described here.

It was thought (Ebling & Rook, 1972) that most pedigrees extended to no more than two generations because marriage rate and fertility are low. Parents with Darier's disease in the present study produced significantly more offspring than did unaffected members of the kindred. Svendsen & Albrechtsen (1959) noted similar results and concluded that married patients did not appear to have fewer children than the normal population, but in fact that the opposite appeared to be true. The fact that the Allegheny mountain region, throughout which the members of this kindred were located, has one of the lowest average amounts of sunlight in the contiguous forty-eight states (Fry, 1963) may contribute to their reproductive success. Since sun exposure is known to aggravate the symptoms of Darier's disease (Kesten & Slatkin, 1953; Preissman, 1946), the reduced sunlight in the area where most members of this kindred reside may reduce the severity of the symptoms and increase the likelihood of finding a marriage partner.

ACKNOWLEDGMENTS

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British Journal of Dermatology (1977) **97,** 341.

Correspondence

SIR, Recently you were kind enough to publish an article of mine on the lines of Blaschko (*British Journal of Dermatology*, 1976, **96,** 349). Since then I have obtained considerable biographical material on Dr Alfred Blaschko from his son Dr Hermann Blaschko of Oxford and it occurred to me that your readers might like to share this information with me.

Alfred Blaschko (Fig. 1) was born in Freienwalde in 1858 and died in Berlin in 1922. His father was a physician. Blaschko was a private practitioner in Berlin; he was not attached to any university.

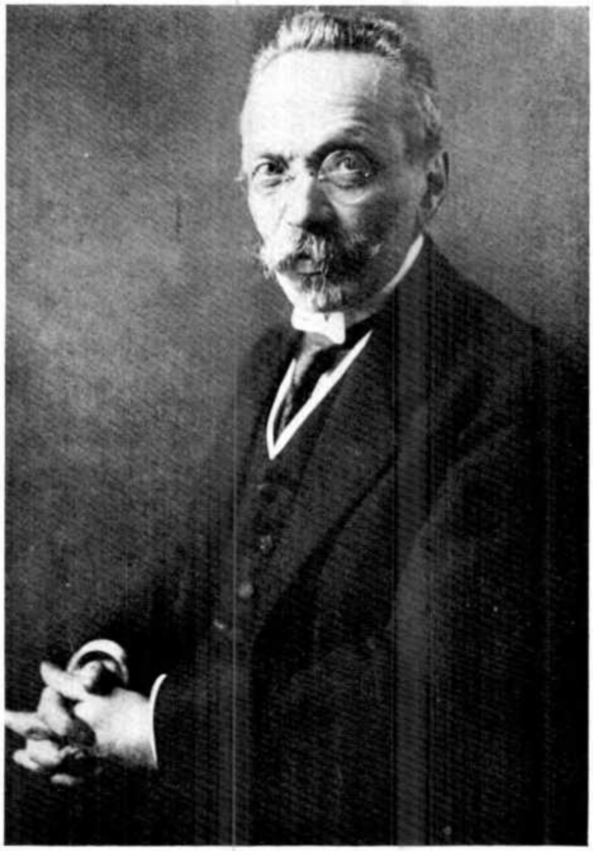

FIGURE 1. Alfred Blaschko

Based on his publications his dermatology interests included, in addition to his study of Blaschko's lines, dermatitis herpetiformis, leprosy, and occupational skin diseases. In the early 1920's his interest switched to social hygiene and he became a strong advocate on the regularization of prostitutes and in 1902 founded the 'German Society for Fight against Venereal Disease'. He was also a contributor and supporter to the Journal of Social Hygiene. In reading his articles on these subjects one receives the impression that he was one of the first advocates of the women's liberation movement.

Unfortunately the hurried departure of his widow from Germany in 1939 did not permit the bringing out of all of his possessions. I have not as yet been able to find the location of the statues which were in his Berlin office and on which he traced his well known lines.

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Immunoelectron microscopy of herpes gestationis

SIR, We were interested in the communication on the detection by immunoelectron microscopy of immunoglobulin G deposits in the skin of immunofluorescence negative herpes gestationis (Holubar, Konrad & Stingl, 1977). We wish to report the same findings in the involved skin of a patient with herpes gestationis.

Direct immunofluorescence of an involved area of skin showed marked linear staining of the basement membrane zone of the epidermis with anti-C3 but with anti-IgG this was negative. Using the horseradish anti-horseradish peroxidase (HRP) multistep technique for immunoelectron microscopy (Holubar *et al.*, 1975), with an antibody to HRP provided by Professor Holubar, we have demonstrated the presence of IgG deposits at the basal lamina. The reaction product was mainly associated with the cytomembranes of the basal cells, particularly at the hemi-desmosomes. This is the identical location for the *in vivo* deposition of IgG as seen in bullous pemphigoid and it seems likely that herpes gestationis is a variant of bullous pemphigoid.

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Book Reviews

Dermatologic Radiotherapy. OTTO BRAUN-FALCO, STEFEN LUKACS & HERBEBT GOLDSCHMIDT (1977) Berlin: Springer Verlag. pp.154. 48 figs. incl. 16 col. plates. Price Cloth DM35,40, US$14.50.

This slim 150 page book with an entirely inappropriate cover illustration is a translation by Herbert Goldschmidt of the updated Dermatologische Rontgentherapie by Otto Braun-Falco and Stefan Lukacs. The two main aspects of this subject are dealt with variably. The chapters dealing with physical principles of production and absorption of irradiation are excellent. There is a clear simplified description of basic methods of radiation dosimetry and practical details on the selection of physical treatment factors. The line drawings are succinct and lucid. Indeed the sections covering the physical basis of dermatological radiotherapy and the general radiobiology chapter should be required reading for dermatologists who intend to use irradiation at any stage in their career.

Unfortunately the chapters dealing with the use of irradiation in benign and malignant dermatoses potentiate many of the old wives' tales associated with fringe dermatological radiotherapy. Concentration on the disease groups where irradiation is of definite value would have been more helpful than mentioning the many diseases where 'response to radiation therapy is not unequivocal'. The dose/time fractionation is inadequately stated and would make a radiotherapist shudder; the value of electron beam therapy is overlooked and there is no attempt to standardize treatment with respect to pathology. The translation betrays the German origin of this book and its American intentions.

In a recent survey in North America 44% of 2444 dermatologists replying to a questionnaire confirmed that they regularly used radiotherapy for the treatment of skin conditions. Although this practice is less common in Great Britain, in the Antipodes the figure may be higher. Thus if this potentially dangerous modality is to be used to best effect a thorough knowledge of the basic principles is necessary. With its inaccuracies and inadequacies this is the best book available to deal with this much maligned and ill-understood sub-speciality and should be read by all dermatologists who will employ this modality.

M.F.SPITTLE

Burckhardt's Atlas and Manual of Dermatology and Venereology: Third American Edition. Edited by P.J.LYNCH & S.EPSTEIN (1977) Baltimore: The Williams and Wilkins Company. Pp. 271, 111 colour plates, 67 black and white. Price £25.70.

This short book is the third American Edition of *Burckhardt's Atlas und Praktim der Dermatologie and Venerologie* 1972: Urban and Schwarzenberg. The authors state that it is not just a translation of the original text of the tenth German edition, but that it has been completely re-written. However, the colour plates and many of the black-and-white photographs have been reproduced from it.

The book is beautifully presented and it is a pleasure to read. The illustrations are all together at the back of the book which could be irritating for a newcomer to Dermatology. The colour plates are of superb quality. The black-and-white photographs are mostly good, but a few are of poor quality. Some of the pictures are rather gross examples of the condition, and would benefit by being less dramatic!

The text is well laid out. The 2 page chapter on Anatomy and Physiology is too brief to be useful. A short account of immunofluorescent techniques and the use of the monochromator would have been useful. The text is impressively up-to-date and is mostly accurate. A few statements might be challenged e.g. 'subcorneal pustular dermatosis and herpes gestationis are probably variants of dermatitis herpetiformis' and 'systemic steroids are usually used in Henoch-Schoenlein purpura'; topical corticosteroids with polythene occlusion are recommended for the treatment of psoriasis. It is stated that side effects of topical corticosteroids are infrequent and usually mild. This is not entirely the experience of Dermatologists in the United Kingdom.

This book will appeal widely to newcomers in Dermatology. Its high price is due to the magnificent colour plates, but it makes it unlikely that it will sell extensively in the United Kingdom.

ANDREW P.WARIN

Social and Health Aspects of Sexually Transmitted Diseases. Principle of Control Measures. WORLD HEALTH ORGANIZATION (Public Health papers (65)). Pp.56. Price Sw.fr.8.

This 56 page pocket sized booklet is number 65 in a series of public health papers published by the W.H.O. Its contents are based on technical discussions during the twenty-eighth World Health Assembly in 1975. Six contributors are mentioned, the British representative being Dr Richard Willcox.

While written in readily intelligible English it does have a flat and impersonal style, inevitable in such a publication, and is inclined to be repetitive. On the second page of text condyloma acuminata aroused misgivings; apart from this, there was a high standard of accuracy in the text.

This publication will be of interest to laymen, ancillary health workers and nurses, but could be read with profit by medical practitioners in many disciplines. A specialist in genital medicine should find little here that is new to him, but there is much which ought to be familiar, but I fear is not, to dermatologists, gynaecologists and other specialists who share common ground with specialists in sexually transmitted diseases.

Although in no sense a text-book, it does contain a large amount of clinical and pathological information. Mention is made of the absence of reliable and comparable statistics resulting from different organization from country to country. China claims to have abolished V.D. So did U.S.S.R. some 25 years ago, since when Soviet authors have contributed many articles to the British Journal of Venereal Diseases! There is discussion on such matters as compulsory notification and control, but no dogmatic conclusions are drawn.

Brief reference is made to interesting matters of contemporary discussion, such as the relationship of herpes virus 2 to cervical cancer, and the persistence of treponemata in treated syphilis. Possible transference of complete penicillin resistance from some strains of *N. gonorrhoeae* to *N. meningitidis* is not mentioned.

There are excellent summaries of current diagnostic techniques and of treatment schedules.

In this country we are inclined to take for granted the facilities of our genital medicine clinics. Dermatologists who have not worked in the field are often unaware of the wide scope and fascination of that speciality.

Now that specialist registration in Europe is of current concern dermatologists in Britain might well acquaint themselves with this booklet. They should make it required reading for their juniors in training.

D. SHARVILL

British Medical Bulletin. Volume 33, No. 2, May 1977. *Somatic and Visceral Sensory Mechanisms*

This volume brings together a series of expert reviews on subjects relating to sensation. They deal systematically with cutaneous and subcutaneous sense organs, cutaneous axons and sensory neurones in the spinal cord, somaesthetic pathways, sub-cortical mechanisms and the sensory cortex. The later chapters, reviewing sensation from muscle, abdominal and thoracic organs, are of less interest to the dermatologist. The general standard is very high and the reviews are written so clearly that they can be understood even by those not well versed in modern neurology. It is a pity that itching, so important to the dermatologist, is dismissed in three lines in a volume running to ninety large pages. I enjoyed reading this volume despite a shuddering sensation, not described in the text either, induced by the increasing complexity of the subject.

J.A.SAVIN

Principal Drugs. S.J.HOPKINS (1977) 5th Ed. London: Faber. Pp. 157. Price £1.50.

The many editions and reprints of this little pocket book over the last 19 years attest to its value. It is intended as a concise guide to all drugs in general use and is directed particularly to nurses but others will find this a valuable aide memoire. 43 pages are devoted to a useful compilation of a number of drugs with other proprietary equivalents. Mr Hopkins' long experience as Chief and now Consultant Pharmacist to Addenbrooke's Hospital allows him to write with great authority and there is little that can be faulted.

I know how easy it is to criticize the omissions of this or that; I did not, for instance, see urea mentioned as a constituent of topical emollients or triamcinolone for intra-articular or intralesional use. But everything else seems to be there and a surprising amount of information is compressed into this small volume which, unlike others, is truly a 'pocket' book.

This volume will follow its predecessors as a valuable guide to the increasing complexity of pharmaceutical products and nomenclature. Doctors, as well as nurses, will want to keep it, if not in their pockets, at least at their elbows.

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Review

Leprosy reactional states and their treatment

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THE DISEASE SPECTRUM

Leprosy is a disease of low infectivity demonstrating a spectrum of clinical, histopathological and immunological characteristics. At one end of the spectrum is lepromatous leprosy (LL), the clinical features of which are bilaterally symmetrical macules, papules or nodules, or a combination of all three. The histopathological picture is of numerous macrophagic granulomata containing globi of viable bacilli, no epithelioid cells and only very few lymphocytes, separated from the epidermis by a clear subepidermal zone. There is a specific defect in cell-mediated immunity to the bacillus which, being a facultative intracellular parasite, cannot therefore be eradicated from the body despite a normal humoral antibody response. Split skin smears reveal abundant bacilli and the Mitsuda (lepromin) test is negative. Lepromatous leprosy can be subdivided into polar (LLp) and sub-polar (LLs), the latter being less stable immunologically as it has evolved from an earlier borderline phase; the earlier name for this sub-type was indefinite lepromatous (LI). At the other end of the spectrum the clinical picture is of a single erythematous, cutaneous plaque with a raised distinct margin and/or thickening of a single peripheral nerve with associated sensory or motor dysfunction. Histopathological examination reveals few epithelioid granulomata encroaching upon or involving the basal epidermal layer with no visible bacteria and numerous small lymphocytes. There is no depression of cell-mediated immunity so no bacilli are found in split skin smears and the Mitsuda reaction is strongly positive, often with central cutaneous necrosis. This is known as tuberculoid leprosy (TT).

Between these two poles exists a continuous spectrum of changes known as borderline leprosy. There exists also a clinical and histopathological state known as indeterminate leprosy which cannot be fitted into the framework described but which does warrant therapy and careful follow up as it can, if left untreated, break into the spectrum at any level and at any time. It should be admitted however that this form of leprosy can, and frequently does, regress spontaneously.

The clinical state of a patient can migrate to and fro along this spectrum, being most stable at the two polar ends (LLp and TT) and most labile in mid zone (BB). The internationally accepted classification of Ridley & Jopling (1966) and Ridley & Waters (1969), which has recently been revised by Ridley (1974), defines four intermediate, arbitrary stages between the two polar forms. These have distinct clinical and histopathological features and are known as:

Sub-polar lepromatous (LLs); Borderline lepromatous (BL); Borderline (BB); Borderline tuberculoid (BT).

The reader is referred to the original works for further details of this subject.

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Leprosy reactions

Abrupt changes in clinical stability occur in leprosy and are known as 'reactions'. The majority of these reactions may be classified into two aetiological groups (Waters, Turk & Wemambu, 1971). The principle points of differentiation are listed in Table 1.

TYPE 1 (LEPRA) REACTION

The commonest form of reaction is known as the Lepra or Type I reaction and patients in non-polar categories of leprosy are at risk whether treated or untreated. Evidence that this reaction is caused by alteration in cell-mediated immunity is provided by the following: (1) Changes in the Mitsuda reaction. (2) Altered reactivity of lymphocyte transformation tests using whole *M.leprae* as antigen (Barnetson, Pearson & Rees, 1976). (3) Histopathological evidence in reactive skin and nerve lesions of changes in the numbers of defensive cells such as lymphocytes, epithelioid and giant cells, and changes in the numbers of viable bacilli (Ridley, 1969). (4) Lymph node histological evidence of changes in the cell types occupying the T cell, paracortical areas. In LLp and LLs the normal lymphocyte population is replaced by macrophages full of bacteria. In reversal reactions (see below) the lymphocytes reappear, suggesting return of T cell activity (Turk & Waters, 1971).

TABLE 1. The two main aetiological groups of 'reaction' in leprosy

	Type 1 reaction	Type 2 reaction
Type of leprosy involved	Whole of leprosy spectrum except the polar forms (LLp & TT)	Lepromatous (LLp & LLs) and occasionally borderline lepromatous (BL)
Aetiology	Changes in cell-mediated immunity against *M. leprae*	Immune complex disease
Relationship of reaction to therapy	Treated patients suffer 'Reversal' and untreated patients suffer 'Downgrading' reactions	Majority of patients are on therapy. 50% of susceptible group are affected by the end of first year of sulphone therapy
Histology	There is a shift of classification. Treated toward tuberculoid, untreated toward lepromatous	Existing leprosy lesions show some oedema. ENL lesions show a vasculitis with polymorphonuclear cell infiltrate and scanty fragmented bacilli
Clinical features	Swelling of the existing skin and nerve lesions. In severe reaction constitutional upset, cutaneous ulceration and neural abscesses can occur	No change in morphology of existing lesions. Fever and malaise are the norm. Complications include ENL, neuritis, orchitis, iritis, epistaxis and depression
Suppression therapy	Steroids Clofazimine	Steroids Thalidomide Clofazimine

Effective therapy of borderline leprosy results in reduction of the antigenic load and the patient is able to strengthen his ability to produce delayed hypersensitivity at the sites of residual bacterial deposition in the skin and peripheral nerves. This results in a shift along the clinical spectrum LLs to BT with subsequent improvement of the patients' ability to contain the infection. This event usually occurs within the first year of therapy, especially in cases of BL and BB leprosy, and is known as a 'reversal' reaction. It is so called because it is a reversal of the normal tendency of untreated leprosy spontaneously to downgrade towards the lepromatous end of the spectrum. This 'downgrading' reaction may be stimulated by such factors as intercurrent infection or disease, nutritional and hormonal factors but more frequently it occurs for no apparent reason. The antigenic load is increased and cell mediated immunity is depressed with resultant reduction of reactivity to the bacillus and increase in infectivity. Clinical features are similar for both forms of Lepra reaction and only by histological methods and by observation of altered Mitsuda reactivity can the clinician be certain which way the disease is progressing. There is pronounced increase in the erythema of existing skin lesions, and swelling and tenderness of the peripheral nerves. In severe reactions there may be fever, malaise and generalized oedema. The sudden and severe nerve damage so often produced leads to profound morbidity from paralysis of sensory and motor function. Thus, although the reversal reaction produces improved resistance to *M. leprae*, it can do so at great cost to the patients' appearance and functional ability.

Type 2 (ENL) reaction

The second type of reaction usually manifests itself by the end of the first year of sulphone therapy in about 50% of LLp and LLs, and less frequently in BL cases. This is the so called erythema nodosum leprosum (ENL) or Type 2 reaction. Other provoking factors include intercurrent infection, physical or mental stress, pregnancy, parturition and vaccination, especially successful smallpox vaccination. Rea & Levan (1975) reviewed 32 patients with this form of reaction and less than a third of them had received any form of anti-leprosy therapy prior to its onset. They stressed that this reaction is a manifestation of the disease and not always a complication of its therapy. Great diagnostic difficulties can be caused, especially in non-endemic areas such as Britain, by new cases of leprosy presenting in Type 2 reaction. They can present in extraordinary ways, often to specialist clinics, with resultant long delays before the true diagnosis is considered (Powell & McDougall, 1974; McDougall & Archibald, 1977). The terminology stems from the commonest physical sign of this reaction which is a form of erythema nodosum. In 1959 Jopling introduced the term Type 2 reaction in order to encompass the clinical spectrum of the reaction without concentrating on any one manifestation, such as ENL, which need not be present for the diagnosis to be made.

The commonest mode of presentation is the appearance of crops of tender, erythematous papules or nodules on normal skin between existing lepromatous lesions that remain morphologically unchanged although the histologist will be able to demonstrate oedema in them. These ENL lesions are evanescent, lasting for only 24–48 h before regressing spontaneously leaving faint bluish hyperpigmentation in the skin. In severe reactions these lesions may be vesicular and may break down (erythema necroticans). The lesions may also take the form of typical target lesions of erythema multiforme, they may be haemorrhagic and, perhaps most importantly of all, they may never appear. Cropping can continue for days, weeks or years and only the hairy scalp, axillae, groins and perineum seem immune from involvement.

Systemic disturbance is the rule with intermittent fever and continuous malaise. In addition any, all or none of the following manifestations may occur: Generalized or dependent oedema, neuritis, bone and joint pains, arthrosis, rhinitis, epistaxis, acute epididymo-orchitis, proteinuria, acute iridocyclitis, and swollen and tender regional lymph nodes. Insomnia and depression are common and can lead to a suicidal attempt. Without adequate treatment iritis can lead to blindness, and testicular damage may result in sterility, gynaecomastia and other endocrine disturbances.

A different form of Type 2 reaction, known as Lucio's phenomenon, occurs in some parts of Mexico and Central America as a complication of the diffuse lepromatous leprosy of Lucio and Latapi. Interested readers are referred to the paper by Latapi & Chevez Zamora (1948) which cites the original writings of Lucio & Alvarado of 1852.

Whereas Type I reaction is a manifestation of changes in CMI and a lymphocytic reaction, the skin lesions of Type 2 reaction are characterized by intense perivascular, bacteria-free polymorphonuclear infiltrations reminiscent of the Arthus reaction seen in experimental animals. There is also an intense vasculitis with fibrinoid necrosis in deep dermal vessels and capillary necrosis in more superficial lesions (Ridley, 1969). Humoral antibody production at the lepromatous end of the spectrum is well developed and antibody against mycobacterial antigen can be detected in large quantities (Rees *et al.*, 1965). Other autoantibodies also abound giving positive rheumatoid and antinuclear factor and false positive Wasserman reactions. There is also a hypergammaglobulinaemia with raised levels of IgM, IgG and C3 (Bonomo & Dammacco, 1968).

Fall of the viable bacterial load either as a result of sulphone therapy or for the other reasons mentioned above is associated with release of soluble antigenic material at the sites of bacterial degradation; this has been demonstrated using powerful antimycobacterial antisera (Rees *et al.*, 1965). Immunofluorescence studies on biopsy material from skin lesions in Type 2 reactions have shown granular deposits of immunoglobulin and C3 (BIc/BIa) in the polymorphonuclear infiltrates of the dermis in areas unrelated to aggregates of bacteria, but there is as yet no evidence as to the exact nature of the soluble antigen in these immune complexes (Wemambu *et al.*, 1969; Waters *et al.*, 1971).

The widespread systemic manifestations of this reaction suggest the existence of circulating immune complexes in addition to the fixed complexes in the skin. This reasoning is strengthened by the demonstration of circulating CIq precipitates that may be immune complexes (Gelber *et al.*, 1974), and the presence of renal lesions consistent with the pathology of immune complex glomerulonephritis in patients with Type 2 reaction (Shwe, 1971; Drutz & Gutman, 1973; Bullock, Callerame & Panner, 1974).

THERAPY OF REACTIONS

General principles

Principles of chemotherapy of bacterial infections dictate that medication be directed at eradication of the infecting organism and leprosy is no exception. If we ignore for the moment the very real existence of dapsone resistant organisms then the treatment of choice for leprosy is dapsone. Despite previous thoughts on the subject there is in 1977 ample evidence that there is never any indication to stop such therapy or to reduce the dosage to below 1–2 mg/kg/day during reactional states (Waters & Helmy, 1974). To do so is to encourage proliferation of the bacillus and so prolong the infection and risk the emergence of dapsone resistant strains. These statements are made on the assumption that the clinician has the necessary drugs with which to treat reactional states when and if they occur.

Type 1 (Lepra) reaction

The risk of reversal reactions was thought to be reduced if dapsone therapy was initiated in low dosage in borderline leprosy (WHO Expert Committee, 1966) but until recently there had been no controlled study to support or refute this hypothesis. Barneston *et al.* (1976) reported such a trial and showed that dapsone given in dosage of 50 mg/day produced significantly less reversal reactions than dapsone given in dosage of 5 mg/day. They concluded that as the MID of dapsone has been shown to be about 1 mg daily (Waters, Rees & Ellard, 1968) this result was unlikely to be due to variation in bacterial activity. Dapsone probably has some immunological activity but they were unable to comment further. Certainly they showed no variation in lymphocyte ability to transform to PHA in the two dose regimens.

Once a reaction has developed, urgent therapy is indicated and the most effective form of treatment available is prednisolone initially in a dose of 20–30 mg/day gradually reducing after a few days to the lowest daily dose which will control the most troublesome manifestations. As the natural history of these reactions can span weeks and months so also must the steroid cover be continued with all the risks involved. The side effects of prednisolone will not be considered further in this paper.

An alternative drug is clofazimine (Lamprene) which is a synthetic substituted riminophenazine dye having bacteriostatic properties of the same order as dapsone as well as having anti-inflammatory properties that can prevent or suppress both types of leprosy reaction. Resistance to clofazimine by *M. leprae* has not been reported and so it is commonly used alone as the second line drug for leprosy treatment. Being less likely to initiate reactional states, it is also frequently used as first line treatment in borderline patients with erythematous lesions as these are the patients most likely to develop early Type I reactions if treated with dapsone. Given as a reaction suppressant the effect is somewhat dose related and slower in onset than that produced by prednisolone. It is therefore normal to use prednisolone initially and then to wean the patients off this preparation with clofazimine. The dosage required is usually in the order of 300 mg daily initially but should not exceed 400–500 mg daily (Working Party, 1969).

Apart from the slow onset of effect the disadvantages of clofazimine are mainly its side effects. Perhaps the most prominent of these is the pigmentation produced in the skin and sclera generally and in the leprosy lesions in particular (Browne, 1965). This red-black, melanin produced pigmentation may well be tolerated by naturally pigmented patients who know the agonies of severe reactions but it can be very disfiguring in light skinned patients. The jet black pigmentation at the sites of active leprosy lesions in Caucasian skin can persist for years after clinical 'cure' has been effected (Jopling, 1976a). Other side effects are red colouration of urine, stools, sputum and sweat, dryness of skin with ichthyosis of forearms and lower legs, and gastro-intestinal upset (Jopling, 1976b).

Other anti-inflammatory drugs and the trivalent organic antimony compound, Stibophen, are used in the treatment of both types of reaction but they are inferior to both prednisolone and clofazimine. Thalidomide is of no use in the treatment of Type I reactions. Reassurance, analgesics and tranquillisers are of course essential but bed rest should be avoided and light duties encouraged except in severe reactional states.

Surgical decompression of acutely swollen nerve trunks can relieve pain and prevent long-term neuromuscular damage. Palande (1976) described clinical and laboratory tests which, when positive, he believes indicate that surgical intervention is likely to be beneficial. His paper also contains a good review of current neurosurgical opinion.

Type 2 (ENL) reaction

As with Type I reactions there is no indication ever to suspend dapsone therapy or to reduce the dosage below 1–2 mg/kg/day in the event of a Type 2 reaction. It is also important to commence effective antibacterial therapy if a patient presents as a new case of leprosy suffering from such a reaction. Evidence to support these statements includes the following observations:

(1) There is no evidence that the incidence and intensity of ENL is less with low rather than high dose dapsone (Waters, Rees & Sutherland, 1967).

(2) ENL is not always aborted by stopping dapsone and if it is subsequently restarted immediate worsening of the reaction is not produced (Pearson & Helmy, 1973).

Dapsone has been shown to have a beneficial effect in the treatment of experimentally produced Arthus reactions in guinea-pigs (Thompson & Souhami, 1975) and it may be that it has some beneficial effect on the Arthus reaction of ENL. If simple measures such as rest and reassurance are insufficient to control the reaction, and if complications such as iritis and neuritis develop, then additional therapy is urgently indicated.

Prednisolone will rapidly control ENL but high doses are often required for months or even years with the resultant side effects. Initial dosage is usually between 20 and 60 mg daily.

Clofazimine will also control ENL but as in Type I reactions the onset of effective control is slow compared to prednisolone. The dosage regimen, benefits and disadvantages are as previously stated. Perhaps the most useful drug to control this problem is thalidomide. Used in men and post menopausal or sterilized women it is safe and virtually without side effect. It is rapid in onset of effect in the same order as prednisolone although there is no dose for dose relationship with the latter preparation. It has immunosuppressive effects as shown by its effect on survival of skin homografts in rabbits (Bore & Scothorne, 1966) but its exact mode of action in suppression of ENL is unknown. It has been shown to stabilize lysosomal membrane (Hastings, 1974) and to improve motor conduction velocities in nerves involved in ENL (Sheskin, 1975). Its excellent therapeutic effect is well documented (Sheskin, 1965; Pearson & Vedagiri, 1969; Waters, 1971) and the author has had personal experience of its efficacy.

Optimal initial dosage is about 400 mg daily (6 mg/kg) in divided doses and maintenance dose is in the order of 100–200 mg daily. If it is initiated as a substitute for prednisolone it should be given in full therapeutic dosage and the steroid gradually tailed off. Side effects are minimal and are reported as being drowsiness and occasional mild allergic dermatitis, easily controlled with antihistamines. There has been no evidence of the severe sensory neurotoxicity reported by Fullerton & Kremer (1961) who were treating non-leprosy patients. In fact the drug seems particularly useful in treating ENL neuritis (Sheskin, Magora & Sagher, 1969; Waters, 1971).

Thalidomide and dapsone is a fast, efficient and safe combination for treating Type 2 reactions in all except women capable of child-bearing and should be made more freely available to all leprologists.

The acute iritis so commonly seen in this reaction responds slowly to systemic medication and severe cases can lead to scarring and blindness if local measures are not painstakingly applied. Jopling (1971) stressed the importance of instilling steroid eye drops hourly, rather than thrice daily, with a single application of steroid ointment to cover the night; homatropine 1% drops twice daily are given concurrently.

CONCLUSION

Leprosy reactions can be broadly classified into two aetiological groups. Type I (Lepra) reaction is the result of changes in cell-mediated immunity and Type 2 (ENL) reaction is probably due to formation of immune complexes. Therapy must at all times include effective antibacterial drugs to which specific reaction suppressants should be added. Prednisolone and clofazimine are effective in suppressing both types of reaction and thalidomide only in the treatment of Type 2 reaction.

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British Journal of Dermatology (1977) **97**, 353.

News and Notices

University of Cambridge School of Clinical Medicine

The Annual Introductory Course on the Biology of the Skin will be held in Fitzwilliam College, Cambridge on 8–11 December, 1977.

Details may be obtained from the Secretary, Cambridge University School of Clinical Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

University of Cambridge School of Clinical Medicine

The 5th five-year course 'Progress in Biological Sciences in Relation to Dermatology' will be held in Cambridge, 25–29 September 1978.

Details may be obtained in due course from the Secretary, Cambridge University School of Clinical Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

Psoriasis Research Award for 1977

The Department of Dermatology, Baylor College of Medicine, Houston, announces the Mr and Mrs J.N.Taub International Memorial Award for Psoriasis Research for 1977.

This annual award of one thousand dollars was established in 1957 by Carol and Henry J.N.Taub in honour of their grandparents, Mr and Mrs J.N.Taub. Their establishment carried with it the hope that the award would be an incentive to develop a cure to alleviate one of the problems of mankind.

The award is designed for the individual or group whose investigative work in this field has been the most outstanding. Names of applicants for the award, together with supporting data, should be submitted for consideration by 1 December. Results of the research must have been presented before a dermatological medical society or published in a recognized medical journal.

The award's recipient will be determined by a committee of seven men. If, in the committee's opinion, none of the applications reflect outstanding research, no award will be given for the year.

Address all communications to: Dr John M.Knox, Professor and Chairman, Department of Dermatology, Baylor College of Medicine, Houston, Texas 77030, U.S.A.

The Third International Symposium on Infectious Diseases

This symposium honouring Dr S.Krugman, M.D., will be held in Guadalajara, Jalisco, Mexico, on 31 October–2 November 1977. It is sponsored by Universidad Autonoma de Guadalajara and Asociación Mexicana de Infectologia.

For further information write to: Educación Continuada U A G, Montevideo # 3331, Acueducto Providencia, Guadalajara, Jalisco, Mexico.

Sixth Annual Consultant's Course in Dermatology 28–31 March, 1978

This programme will be an intensive course directed to problems in dermatology as seen in practices having a significant number of consultant type patients. It will be held at the University of California, San Francisco.

For further information please contact: Extended Programs in Medical Education, University of California, Room 569-U, San Francisco, California, 94143, U.S.A. or call (415) 666-4251.

Photochemotherapy for Psoriasis

Facilities for the treatment of psoriasis are now available to private patients who are referred by Consultant Dermatologists.

The Waldmann PUVA 6000 unit, widely acknowledged as the best of its kind, is used. It allows wholebody treatment in the standing position.

A regime has been developed based on a London Teaching Hospital's experience and practice.

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An Honorary Medical Advisory Committee made up of four consultant dermatologists is supervising the professional standards and practice of the clinic.

Dermatologists, who would like to know more about this facility, are asked in the first instance to contact:

Mr A.R. Breckons, Director,
Psoriasis UVA (Medical Referals Only) Ltd.,
20 Harley Street,
LONDON W1.
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Malignant Melanoma of the Skin and Mucous Membrane

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Until recently, a diagnosis of malignant melanoma was regarded as a death sentence. This need no longer be the case, however, if the condition is diagnosed and treated quickly.

The contents of this book will enable the physician to solve several problems, the first being that of obtaining an accurate diagnosis. The clinical presentation and pathology of the disease are dealt with in detail. The choice of treatment and what to do if treatment proves to be a failure, i.e. the management of recurrent or incurable disease, is also covered. The book is soundly based on the writers' extensive experience of malignant melanoma.

Recent Advances in Dermatology — 4

Edited by **Arthur Rock**

406 pages illustrated £12.50 ISBN 0 443 01318 7

The policy on which the previous volume of *Recent Advances in Dermatology* was based has been continued in this new and up-to-date account of diseases common in dermatological practice.

With one exception — the chapter on topical therapy — the topics selected are different from those dealt with in the previous volume so that the two may be regarded as complementary.

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HISTOPATHOLOGY OF THE SKIN

Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour), Lippincott £41.60

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration

More than 20 diseases not mentioned in the fourth edition are described in this edition. Some of them are newly recognized entities, such as disseminated superficial actinic porokeratosis, perforating folliculitis, reactive perforating collagenosis, angiolymphoid hyperplasia with eosinophilia, infantile digital fibromatosis, trichilemmoma, and malignant eccrine poroma. Also several previously known diseases because of their increased interest for the dermatologist have been incorporated, among them regional enteritis (Crohn's disease), Tangier disease, lipogranulomatosis (Farber's disease), and generalized eruptive histiocytoma.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm³	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066, Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 97, Number 3, September 1977

CONTENTS

PAGE

Clinical and Laboratory Investigations

A Study of the *In Vitro* Metabolism of Androgens by Human Scalp and Pubic Skin 237
J.B.HAY

Classification of the Types of Androgenetic Alopecia (Common Baldness) Occurring in the Female Sex 247
ERICH LUDWIG

Vitiligo: a New Classification and Therapy 255
MICHIYUKI KOGA

Depigmented Haloes Associated with the Involution of Flat Warts 263
ALEXANDER BERMAN

Eruptive Naevi: Report of Two Cases, with Enzyme Histochemical, Light and Electron Microscopical Findings 267
R.A.J.EADY, J.J.H.GILKES AND E.WILSON JONES

Light Sensitivity in Mycosis Fungoides 279
G.VOLDEN AND P.O.THUNE

Influence of Wind on Chronic Ultraviolet Light-induced Carcinogenesis 285
DONALD W.OWENS, JOHN M.KNOX, H.T.HUDSON, ANDREW H.RUDOLPH AND DOUGLAS TROLL

Chronic Leg Ulcers Treated by the Pinch Graft Method 289
L.G.MILLARD, M.M.ROBERTS AND M.GATECLIFFE

Psoriasis and Stress 297
R.H.SEVILLE

Cutaneous β-glucuronidase: Cleavage of Mycophenolic Acid by Preparations of Mouse Skin 303
EDWARD C.GOMEZ, J.MICHAELOVER AND PHILLIP FROST

Post-kala-azar Dermal Leishmaniasis 307
H.S.GIRGLA, R.A.MARSDEN, G.M.SINGH AND T.J.RYAN

Evolution of the Lesion of Discoid Lupus Erythematosus: a Comparison with Lichen Planus 313
M.G.DAVIES AND R.MARKS

Case Reports

Generalized Eczéma Craquelé as a Presenting Feature of Lymphoma 323
D.J.BARKER AND J.A.COTTERILL

Acquired Ichthyosis: Multiple Causes for an Acquired Generalized Disturbance in Desquamation 327
P.J.DYKES AND R.MARKS

Darier's Disease: a Kindred with a Large Number of Cases 335
ARTHUR L.BECK, JR, ALFRED F.FINOCCHIO AND JAMES P.WHITE

Correspondence 341

Book Reviews 342

Review Article

Leprosy Reactional States and their Treatment 345
D.S.JOLLIFFE

News and Notices 355